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PII: S0378-8741(19)32644-3

DOI: https://doi.org/10.1016/j.jep.2019.112255

Reference: JEP 112255

To appear in: *Journal of Ethnopharmacology*

Received Date: 4 July 2019

Revised Date: 24 September 2019

Accepted Date: 24 September 2019

Please cite this article as: Yadav, V., Krishnan, A., Vohora, D., A systematic review on *Piper longum* L.: Bridging traditional knowledge and pharmacological evidence for future translational research, *Journal of Ethnopharmacology* (2019), doi: https://doi.org/10.1016/j.jep.2019.112255.

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A systematic review on *Piper longum* L.: Bridging traditional knowledge and pharmacological evidence for future translational research

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Abstract

Ethnopharmacological relevance: *Piper longum*, commonly referred as 'Pippali', has found its traditional use in India, Malaysia, Singapore and other South Asian countries as an analgesic, carminative, anti-diarrhoeic, immunostimulant, post childbirth to check postpartum hemorrhage and to treat asthma, insomnia, dementia, epilepsy, diabetes, rheumatoid arthritis, asthma, spleen disorder, puerperal fever, leprosy etc.

Aim of the review: This review offers essential data focusing on the traditional use, phytochemistry and pharmacological profile of *Piper longum* thereby identifying research gaps and future opportunities for investigation on this plant.

Materials and methods: This systematic survey was accomplished as per the PRISMA guidelines. The information was collected from books, and electronic search (PubMed, Science Direct, Lilca and Scielo) during 1967-2019.

Results: Many phytochemicals have been identified till date, including alkaloids as its major secondary metabolites (piperine and piperlongumine), essential oil, flavonoids and steroids. These exhibit a wide range of activities including anti-inflammatory, analgesic, anti-oxidant, anti-microbial, anti-cancer, anti-parkinsonian, anti-stress, nootropic, anti-epileptic, anti-hyperglycemic, hepatoprotective, anti-hyperlipidemic, anti-platelet, anti-angiogenic, immunomodulatory, anti-arthritic, anti-ulcer, anti-asthmatic, anthelmintic action, anti-amebic, anti-fungal, mosquito larvicidal and anti-snake venom.

Conclusion: Amongst various activities, bioscientific clarification in relation to its ethnopharmacological perspective has been evidenced mainly for anti-amebic, anthelminthic, anti-tumor and as contraceptive and hypoglycemic herb. However, despite traditional claims, insufficient scientific validation for the treatment of insomnia, dementia, epilepsy, rheumatoid arthritis, asthma, spleen disorder, puerperal fever and leprosy, necessitate future investigations in this direction. It is also essential and critical to generate toxicological data and pharmacokinetics on human subjects so as to confirm its conceivable bio-active components in the body.

Keywords: *Piper longum*; Pharmacology; Phytochemistry; Traditional evidence

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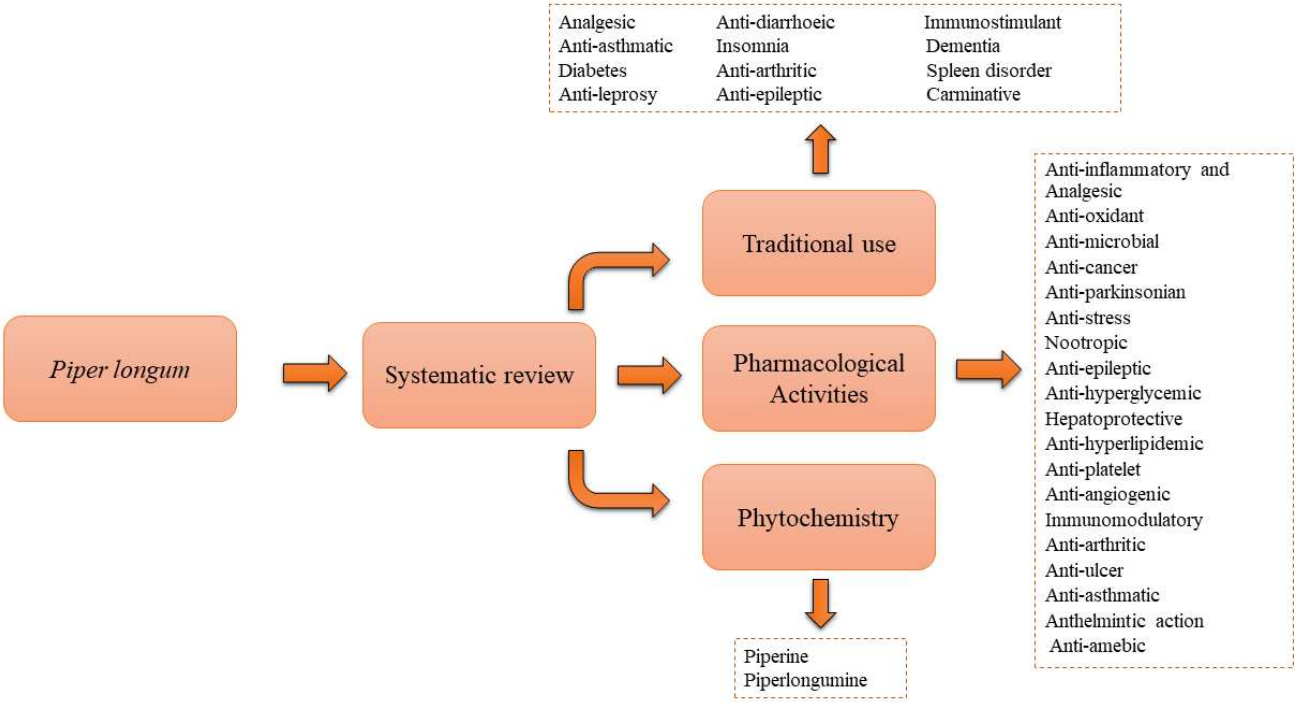
Graphical Abstract:



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List of Abbreviations

5HIAA=5-hydroxyindoleacetic acid
5HT= 5-hydroxytryptamine
6-OHDA=6-hydroxydopamine
ACE=Angiotensin-converting enzyme
AD= Anno domini
ADP=Adenosine diphosphate
ADR=Adriamycin
ALP=Alkaline phosphatase
ALT=Alanine transaminase
ARRIVE=Animal Research Reporting of In Vivo Experiments
AST=Aspartate aminotransferase
BC= Before Christ
CAM=Cell adhesion molecules
cAMP=cyclic AMP
CAT=Catalase
CCl_3·= Trichloromethyl free radical
CCl_4= Carbon tetrachloride
cGMP=cyclic GMP
CH=Cholesterol
COX=Cyclooxygenase
DA=Dopamine
DLA=Dalton's lymphoma ascites
DLS= Dynamic light scattering
DNA=Deoxyribonucleic acid
DOPAC=3,4-Dihydroxyphenylacetic acid
DPPH= 2,2-diphenyl-1-picrylhydrazyl
EAC=Ehrlich ascites carcinoma
ECM=extracellular matrix
FT-IR= Fourier-transform infrared
GABA=Gama amino butyric acid
GM-CSF=Granulocyte-macrophage colony-stimulating factor
GSH=Glutathione
GSH-Px=Glutathione peroxidase
HDL=High density lipoproteins
HMG-CoA=β-Hydroxy β-methylglutaryl-CoA
HPA=Hypothalamus pituitary adrenal
HPLC= High performance liquid chromatography
HVA=Homo vanillic acid
IC_{50}= Half maximal inhibitory concentration
ICAM-1=Intercellular adhesion molecule 1
IL-1β=Interleukin-1β
IL-2=Interleukin-2
IL-6=Interleukin-6
LC50=Minimum lethal concentration 50
LC95=Lethal concentration 95
LDH=Lactate dehydrogenase
LDL=Low density lipoproteins
LPS=Lippoploysacchride
MDA=3,4-Methylenedioxyamphetamine
MIC=Minimum inhibitory concentration
MMP=Matrix metalloproteinases
MPTP=1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH=Nicotinamide adenine dinucleotide phosphate
NF-kB=Nuclear transcription factor-kB
NO=Nitric oxide
NOS=Nitric oxide synthases
NSAID=Nonsteroidal anti-inflammatory drugs
PD= Parkinson's disease
PGD2=Prostaglandins D2
PGE2=Prostaglandins E2
PGI2=Prostaglandin I2
PRISMA=Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTZ=Pentylenetetrazol
RA=Rheumatoid arthritis
RAAS=Renin-angiotensin-aldosterone system
SEM= Scanning electron microscope
SGOT=Serum glutamic oxaloacetic transaminase
SGPT=Serum glutamic-pyruvic transaminase
SNpc= Substantia nigra pars compacta
SOD=Superoxide dismutase
TG=Triglyceride
TH=Tyrosine hydroxylase
TIMP=Tissue inhibitor of metalloproteinases
TNF-α=Tumor necrosis factor alpha
TPC=Total Protein Content
TXA2=Thromboxane A2

UV= Ultraviolet
VCAM-1=Vascular cell adhesion protein 1
VEGF= Vascular endothelial growth factor
VMA=Vanillyl mandellic acid
WBC=White blood cells
XRD= X-ray powder diffraction

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1. Introduction

About 20,000 plant species are utilized in different traditional medicine system around the globe and are considered as potential agents for the revelation of new medications. The family Piperaceae include 4000 species, divided into 5 genera. The *Piper* (2000 species) and *Peperomia* (1600 species) are the two important genera of this family (Tebbs, 1993). Linnaeus, in his Species plantarum, in the year 1753, recognized 17 species of this family, all of which were included in the genus Piper (Yuncker, 1958). The *Piper* species have high commercial, economical and medicinal importance. Economically, *Piper nigrum* and *Piper longum* are the most important species in this family (Scott et al., 2008). The utilization of *P. longum* is mentioned in the Ayurvedic medicinal system and available literature has recommended unripe fruit of *P. longum* for respiratory disorders, gastro-intestinal disorder, disorders of metabolic imbalance, aphrodisiac, emmenagogue, circulatory stimulant and analgesic (Evans, 2009). *Piper nigrum* and *Piper longum* are known to contain an alkaloid referred as piperine, which gives pungency to this herb. *P. longum* is the famous ingredient in the formulation called 'Trikatu' (meaning three acids) and is known to be a prominent bio-enhancer (Johri and Zutshi, 1992).

The long tradition of use and efficiency of *P. longum* in various disorders and presence of specific alkaloids have made this herb a choice for researchers to validate the scientific actions, although the lack of comprehensive and systematic analysis of the literature on this subject prompted us to gather detailed information about this herb. The previously published reviews provide only a brief glimpse on the pharmacology and phytochemistry of *P. longum* and are also not up-to-date (Khushbu et al., 2011; Srivastava, 2014; Zaveri et al., 2010), nevertheless, network pharmacology approach has been put forward recently to examine its medicinal effects and phytochemistry(Choudhary and Singh, 2017). A complete inspection is required to advance future research on *P. longum*. Thus, this systematic review of literature attempts to review and critically analyse the pharmacological activities reported with this plant available from 1967- to date. The review begins with a section on the traditional uses, history, and botany of *P. longum* followed by critical analysis on various pharmacological/ potential therapeutic actions and phytochemistry of the plant. Finally, we provide research directions regarding the plant. To the best of our knowledge, no review article has previously covered all these aspects. We believe that the present article will provide a better understanding of *P. longum* and its properties. This

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data may be valuable in planning future inspections specifically clinical preliminaries, and in growing new pharmaceuticals containing *P. longum* or its active constituent.

2. Methods

2.1 Search strategy

An orderly survey was executed through a literature search implemented in January 2019, which included articles distributed over a period of 52 years (1967 to 2019). This survey was performed through particular databases (PUBMED, Lilca, Scielo and Science Direct) utilizing diverse mixes of the accompanying catchphrases: *Piper longum*, phytochemistry, pharmacological actions. We did not contact investigators and we did not endeavor to recognize unpublished information. This systematic survey was accomplished as per the criteria depicted on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. No confinements were put on the dates or dialects of the distribution amid research studies. A restricted measure of the manual search was attempted. Likewise, reference records were filtered to recognize other important investigations.

2.2 Study selection

The studies were selected based on the following inclusion criteria: articles reporting pharmacological actions of *P. longum* extracts, articles reporting phytochemistry or isolation of secondary metabolite of *P. longum,* articles and book chapters reporting traditional use and all articles meeting the above criteria and published in English. Other review articles, conferences, editorial/letters, case reports, conference proceedings or articles that did not meet the inclusion criteria were excluded from this systematic review. The preferred articles were physically explored with the reason for distinguishing and barring the works that did not fit the criteria depicted previously.

2.3 Data extraction

All selected manuscripts were analyzed for the year of publication, the country where the research was performed, part of the plant, isolated chemical compounds and evaluated biological activities. For the pharmacological studies, the collected information also included extract or fraction evaluated, experimental model (*in vivo* or *in vitro*), dose or concentration, and results (pharmacological activity verified). All the selected information was composed in tables and figures, as will be exhibited all through this article.

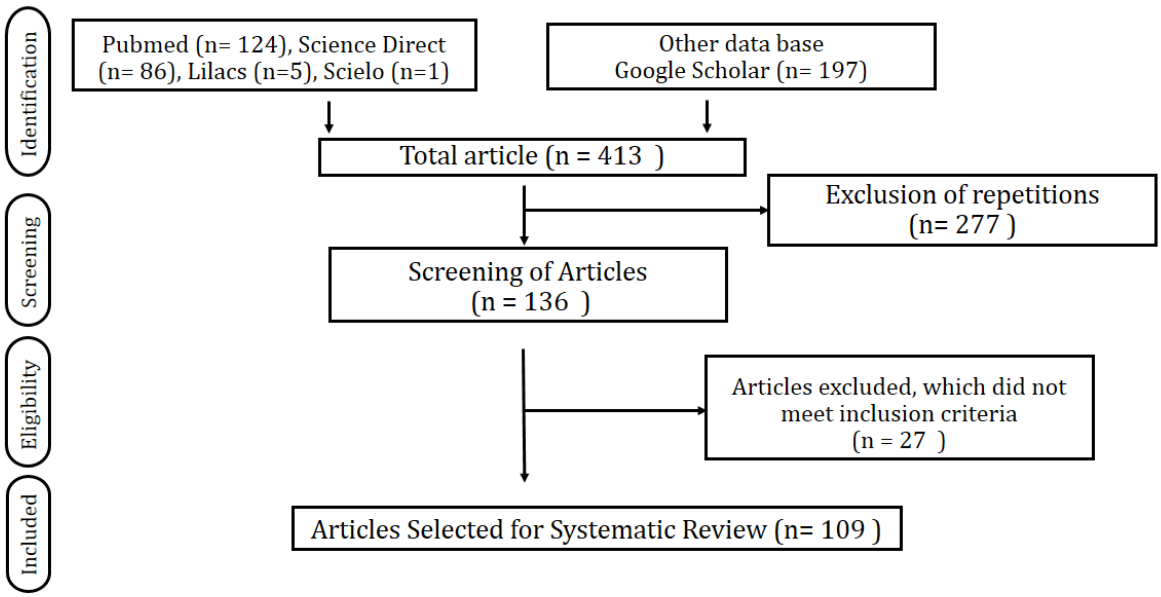
2.4 Methodological quality assessment of pharmacological studies

The risk of bias and quality of the pre-clinical pharmacological studies design were assessed using a checklist, adopted and modified in reference to ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) and Siqueira-Lima et al., 2017 (Siqueira-Lima et al., 2017). For methodological quality assessment, experiments conducted to evaluate the *in vivo* biological action of *P. longum* extracts have been considered. This investigation permitted assessing the methodological quality of the designated studies concerning the randomization of the treatment allocation, blinded drug administration, blinded outcome assessment and outcome measurements.

3. Results and Discussion

Articles screened from primary search leads to identification of total 413 articles, with 124 from PUBMED, 86 from Science Direct, 5 from Lilca and 1 from Scielo, while 197 articles from google scholar. However, most of the articles were indexed in two or more databases and hence to avoid duplication, they were removed resulting in a total of 136 articles. After an initial screening of titles, abstracts, full text and time of publication, 109 articles were selected and included in study, while the remainder did not meet the inclusion criteria. The process of identification and screening of articles for this systematic review has been represented in Figure 1.

Figure 1: Flow chart of included studies



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3.1 History, Traditional uses and Ayurvedic formulation

The foundation of this shrubby plant is utilized restoratively in India, however, its principle article, the spice, consists of the fruit pods. In Sanskrit, it is called pippali, in Chinese as pibo and called long pepper in Europe. *P. longum* is referenced exceptionally in Sanskrit writing, in the Yajurveda and Atharvaveda, gathering of custom and enchanted verse that are perhaps to be dated somewhere close to 1000 and 500 BC. Dried long pepper is classed in Indian spice as a pungent flavor. Long pepper, when fresh, provokes phlegm, is sweet in taste, heavy and oily while when dry, is destructive of phlegm and wind. Being pungent and hot, it is capable of increasing semen. Though food of pungent flavor does not generally increase the semen long pepper and dry ginger are exceptions. For nearly 2400 years, long pepper was one of the most valuable of Indian exports. It had reached Greece, as it was mentioned in Athenian drama, early in 4^{th} century BC. Theophrastus has also described pepper and categorized in two divisions (a) the round one and (b) the other of long appearance; both have heating nature and serve as an antidote for hemlock. Theophrastus also utilized pepper mixed with vinegar to cause a revival in suffocated patients. Pliny, in the first century AD, has also utilized pepper, not only as food but also as medicine, especially as stimulating tonic. They made pepper wine with the addition of honey and other flavorings agents. During medieval times and in 19^{th} century, it was mostly utilized as a spice and as a drug in Europe. Also, in China, Ji Han, Su Gong, and Tia Zong have used it as medicine for curing various digestive ailments (Dalby, 2002; Evans, 2009; McGee, 2004).

The fruits are used as a spice and also in pickles, preservatives, foods, beverages, liquors, and medicines. The most important use of long pepper is as a medicinal ingredient in the Indian systems of medicine – Ayurveda, Siddha, and Unani. Ayurvedic Pharmacopeia has mentioned the use of *P. longum* fruit in abdominal pain, phantom tumor, bronchitis, abdominal disease, rheumatism, leprosy, fever, spleen related disorders and in parasitic infection, while *P. longum* stem and root is indicated in flatulence, phantom tumor, ascites and abdominal enlargement. Also, in folklore medicine, *P. longum* is reputed for treatment of asthma, bronchitis, dysentery, pyrexia and insomnia (Johri and Zutshi, 1992; Khare, 2008). The utility of *P. longum* is mainly because of the piperine content, which itself possesses a diverse range of pharmacological actions (Chinta et al., 2015). *P. longum* is used in 135 Ayurvedic formulations as reported by Kamboj (Kamboj, 2000). The ancient verse of Ayurveda suggests the action of *P. longum* as

bioenhancer and helps in removal of endotoxins from the body. The main part of *P. longum* utilized for medicinal purpose involves fruit and root. Also, among various therapies, Rasayana is an important therapy in Ayurveda, which deals with the promotion of strength and vitality. *P. longum* is one such plant, which is utilized in Rasayana therapy and also, for improvement of cognitive impairment as proposed in 'Charak Samhita'(Manyam, 1999; Ramawat and Goyal, 2008). Ayurvedic Pharmacopeia has stated some of the formulation of *P. longum* fruit as Amrtariasta, Ayaskrti, Cyavanaprasa Avaleha, Gudapippali, Asvagandhadyarista, Kumaryasava, Candanasava, siva Gutika and Kaisora Guggulu; and the formulations of *P. longum* stem as Pancakola Curna, Dasamula Taila, Dasam ulapancakoladi Kvatha Curna and Dasam ulastapalaka Ghrta (The Ayurvedic Pharmacopia of India. Parts II and IV. Ministry of Health and Family Welfare. Dept of AYUSH. 133 and 91).

3.2 Geographical distribution, Botanical classification and description

P. longum is a native of South and South East Asia and the Indo-Malaya region. It is distributed in north-eastern India, from the southern edge of Nepal to Bengal and Assam and also got transplanted to Kerala in the southwest of the peninsula and grows wild there too (Dasgupta and Datta, 1980; Evans, 2009; Wiart, 2013). Further the botanical classification and description of *P. longum* has been depicted in Table No 1.

Table No. 1 Botanical classification and description of *Piper longum* plant

Botanical classification			**Reference**
Family: Piperaceae-Pepper family Genus: *Piper* Species: *P. longum*			United States Department of Agriculture, Natural Resources Conservation Service
S. No.	**Part of Plant**	**Description**	**Reference**
1	Fruits	➢ Greenish-black to black in color, Cylindrical in shape. ➢ Surface of the fruit is rough and composite. ➢ When broken, surface shows a central axis and 6-12 fruitlets arranged around an axis. ➢ The fruit tastes pungent producing numbness on the tongue and odour is aromatic.	Chaveerach et al., 2006; Peter, 2006; Wiart, 2013; Joshi, 1944; Dasgupta and Datta, 1980

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2	Stems	➢ Stems are slender in shape, with aromatic odor. ➢ These are available in cut pieces, having distinct internodes and swollen nodes with a number of small rootlets and root scare; stout and cylindrical. ➢ The color is reddish brown to grey; odour is aromatic with pungent taste.	Chaveerach et al., 2006; Peter, 2006; Wiart, 2013; Joshi, 1944; Dasgupta and Datta, 1980
3	Leaves	➢ Leaves on creeping branch and epiphytic branches have ovate or elliptic blade. ➢ Leaves on free branches have ovate to ovate-oblong blade. ➢ Leaf blade are membranous, dark green. ➢ Leaf apex is acuminate; base is cordate or oblique.	Chaveerach et al., 2006, Peter, 2006; Wiart, 2013; Joshi, 1944; Dasgupta and Datta, 1980
4	Male spikes	➢ Bract is orbicular, stalked with 2 stamens	Evans, 2009; Wiart, 2013; Dasgupta and Datta, 1980
5	Female spikes	➢ Female spike is erect, bract is circular, peltate; with 3 stigmas	Evans, 2009; Wiart, 2013; Dasgupta and Datta, 1980

3.3 Pharmacological Report

P. longum possesses a large number of biological actions which has been evaluated through various *in vivo* and *in vitro* studies. Figure 2 represents number of publications mentioning different pharmacological effects of *P. longum*. It has been observed that amidst the various actions of *P. longum*, the majority of articles are related to its action on central nervous system. Also, most of the studies involving *P. longum* were conducted by research groups located in India and China. This is possibly related to the geographic distribution of *P. longum*, since a large number of species have been found in India and China. Concerning the annual evolution of the publications, a larger number of articles published in 2013 were observed (Figure 3). Regarding the analysis of quality of the studies inserted, it is observed that none of the study shows the sample size calculation for animal study. However, majority of studies clearly defines scientific background, dose, duration, route of administration, evaluating parameters and statistical methods (Figure 4). The below-mentioned text assesses the pharmacological action of *P. longum* extract. The various activities are presented in Table No.2

Fig. 2. Publications for major pharmacological effects of *Piper longum*

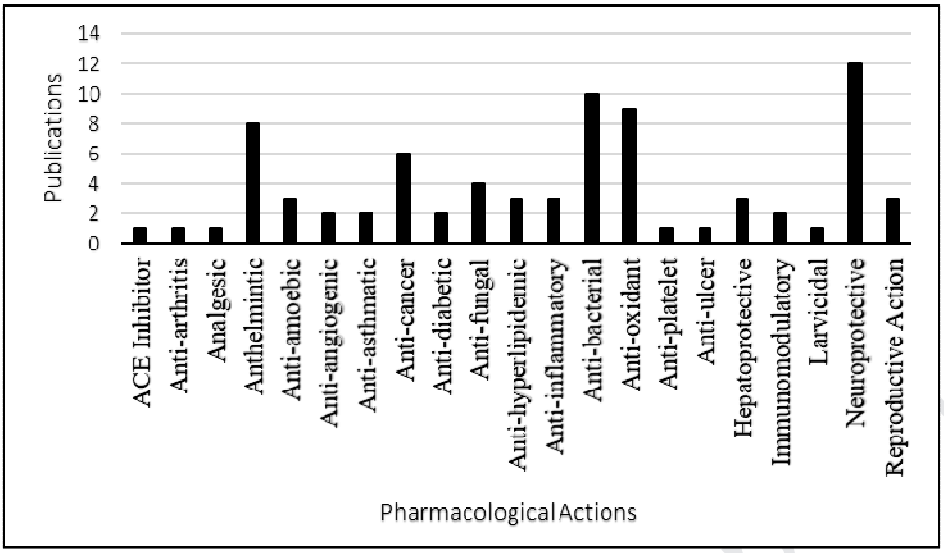


Figure 3: Distribution of the selected manuscripts for the systematic review according to the year of publication

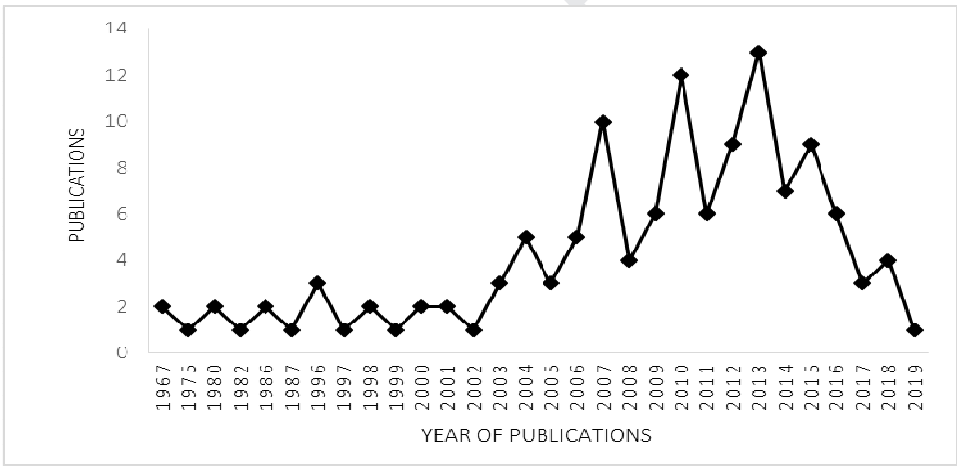
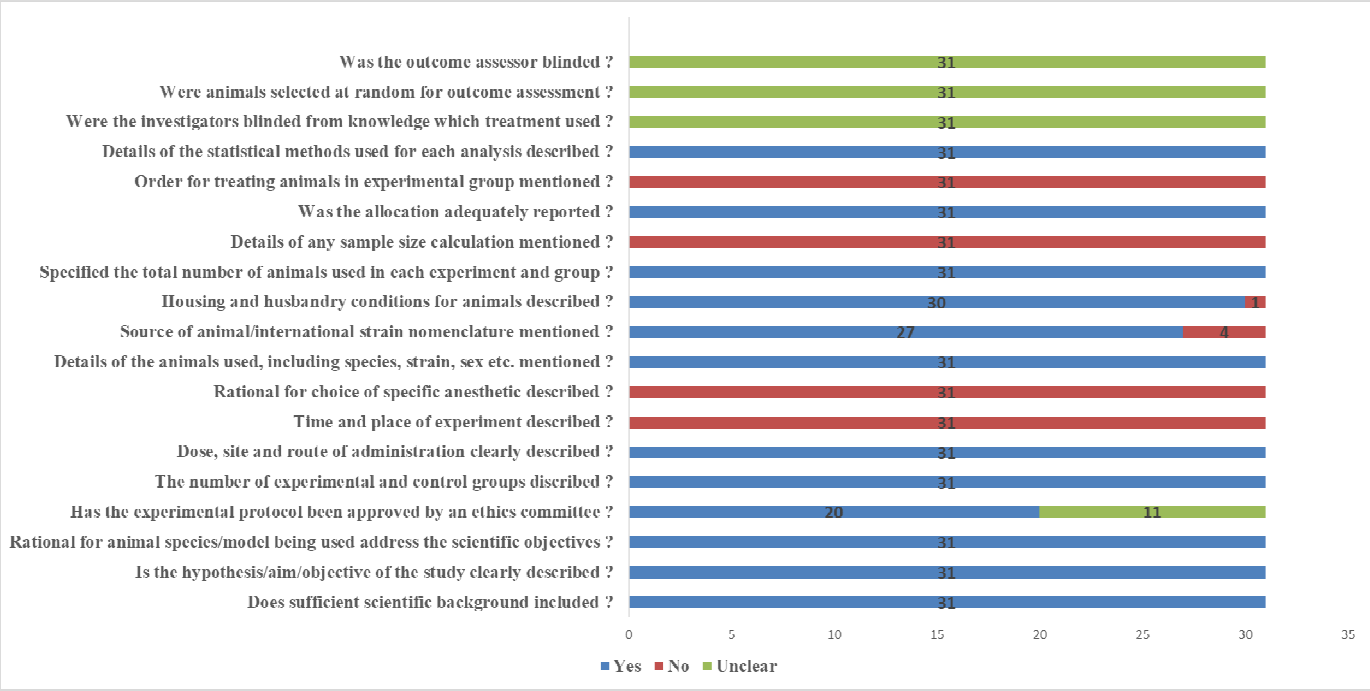


Fig. 4. Evaluation of the methodological quality of the preclinical pharmacological studies on *P.longum* extract included in the review



The criteria for evaluating the methdological quality of pre-clinical pharmacological studies in Figure 1 was suitably modified and derived from ARRIVE Guidelines and from Siqueira-Lima et al., 2017. The bars indicate the proportion of articles found for each criterion adopted. A total of 31 *in vivo* studies on *Piper longum* extract as per inclusion criteria were assessed. Blue bars indicate the proportion of articles that met each criterion, red bars indicate the proportion of studies that did not and green bars indicate the proportion of studies with unclear answers.

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Table No 2: Pharmacological Activities of *Piper longum*

Activity	Animal, Dose, Duration, Route of Administration	Extract	Part	Model	Evaluating Parameters	Major Findings	References
Analgesic	Rats, 200, 400, 800 mg/kg, p.o.	Aqueous	Root	Rat tail flick method, Acetic acid induced writhing test	Reaction time for thermal stimulus, Reduction in number of writhes	Delay in reaction time to thermal stimulus was 6% while 50% protection was offered against writhing (Standard drug- Pentazocine and Ibuprofen)	Vedhanayaki et al. 2003
Anti-inflammatory	2.5, 5, 7.5, 10, 12.5, 15, 17.5 µg/ml	Chloroform	Fruit	*In vitro*, Human umbilical vein endothelial Cells	TNF-α-induced expression of CAM	Treatment inhibits adhesion of neutrophils to endothelial monolayer, Block the TNF-α-induced expression of CAM, Inhibits the NADPH catalyzed rat liver microsomal lipid peroxidation	Singh et al., 2008
	Rats, 300 mg/kg, p.o.	Methanol	Leaf	Carrageenan induced paw edema in rats, Dextran induced paw edema in rats, Cotton pellet induced granuloma in rats	Percentage inhibition in swelling	39.81% inhibition in carrageenan induced edema, while 67.51% inhibition in dextran induced paw edema was observed with treatment, In cotton pellet induced granuloma did not show reduction in swelling (Standard drug- Diclofenac)	Vaghasiya et al., 2007
	Rats, 0.5 and 1 ml/kg, p.o.	Oil	Fruit	Carrageenan-induced right hind paw edema model	Reduction in paw edema	72% reduction of edema induced by carrageenan (Standard drug- Ibuprofen)	Kumar et al., 2009

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	Rats, 200 mg/kg, p.o.	Ethanol	Fruit	Carrageenan-induced paw edema and Formaldehyde induced paw edema	Reduction in paw edema	28% inhibition in swelling of paw induced by carrageenan, 46% inhibition in formaldehyde induced paw edema. (Standard drug- Phenyl butazone, Diclofenac)	Kumari et al., 2012
Anti-microbial	MIC=25 mg/mL	Acetone, Ethanol and Methanol	Fruits	Agar well diffusion method	Zone of inhibition	Treatment was very effective for *C. albicans* (Standard drug- Ciprofloxacin, Amphotericin-B)	Aneja et al., 2010
	400 µg	Petroleum ether, Ethyl acetate, Chloroform and Methanol	Whole plant	Agar well diffusion method	Zone of inhibition	Treatment was effective (Standard drug- Clotrimazole, Kanamycin)	Ali et al., 2007
	MIC = 41.6 and 83.9 mg/ml	Ethanol	Pod	Agar well diffusion	Zone of inhibition	Treatment was effective (Standard drug- Gentamicin)	Arambewela et al., 1999
	1, 5, 25, 50, 100 µg/ml	Methanol	Leaf	Agar well diffusion	Zone of inhibition	Treatment was effective against these bacterial strains (Standard drug- Cefotaxime)	Vaghasiya et al., 2007
	1, 5, 25, 50, 100 µg/ml	CCl4, Benzene, Chloroform, Ethyl acetate, Acetone, Ethanol, Aqueous	Fruits	Agar well diffusion	Zone of inhibition	Treatment was effective as anti-bacterial	Khan and Siddiqui 2007

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Anti-arthritis	Rats, 200, 400 mg/kg, p.o.	Aqueous	Seeds	Freund's complete adjuvant induced arthritis rats	Reduction in paw edema by plethysmometer and radiographic image	Reduced paw swelling (Standard drug- Diclofenac)	Yende et al., 2010
Anti-diabetic	Male wister albino rats, 200 mg/kg, 30 days	Aqueous	Roots	Streptozotocin induced diabetes	Fasting blood glucose, glycosylated haemoglobin	Treatment helps in preventing hyperglycaemia	Nabi et al., 2013
	Albino wistar rats, 100 and 200 mg/kg, 28 days	Essential oil	Fruit	Streptozotocin induced diabetes	Glucose level, Insulin level, Liver glycogen content, Glycosylated haemoglobin	Treatment results in anti-diabetic action	Kumar et al., 2013
Anti-oxidant	100, 200, 400, 600, 800, 1000 µg/ml	Ethanol	Seeds	DPPH assay, Scavenging activities of Super oxide, Nitric oxide, and Hydroxyl radicals	Assay for quenching activity was performed	Treatment show potential anti-oxidant action (Standard drug- Ascorbic acid)	Ramesh et al., 2011
	Rats, 300 mg/kg, p.o., 20 days	Ethanol	Fruit	Monosodium glutamate induced toxicity	GSH, ALT, AST, TG, CH, urea, histological examination	Provide protection to liver and kidney from oxidative stress of monosodium glutamate	Thomas et al., 2009
	Rats, 250, 500 mg/kg, p.o. 21 days	Ethanol	Fruit	Adriamycin induced cardiotoxicity	Aspartate transaminase, Alanine transaminase, Lactate dehydrogenase, Creatine kinase, CAT, SOD, GSH	Protection against ADR induced oxidative stress and reduce the cardiotoxicity due to anti-oxidant potential	Wakade et al., 2008

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	Rats, 100 mg/kg, p.o., 7 days	Petroleum ether	Root	Isoproterenol induced myocardium ischemia	Lipid peroxide, glutathione, DPPH	Potential anti-oxidant action with protection in myocardial ischemic	Jagdale et al., 2009
	Rats, 50 mg/kg, p.o., 14 days	Ethanol	Seeds	Carbontetra chloride induced hepatotoxicity	Total anti-oxidant activity, Inhibitory effects against protein oxidation, SGOT SGPT, ALP, ACP, LDH	Hepatoprotective effect is possibly related to its marked anti-oxidant activity (Standard drug- Silymarin)	Samudram et al., 2009
	50, 100, 250, 500 µg/ml	Hydro alcoholic, Ethyl acetate, Butanol	Fruit		DPPH activity	Anti-oxidant activity was evaluated	Chaudhary et al., 2012
	10, 20, 40, 50 µg/ml	Hexane Methanol Ethanol	Fruit	L6 (Mouse myoblast) and 3T3L1 (Mouse pre-adipocytes)	Flavonoid content, TPC, DPPH scavenging activity, Hydroxyl radical scavenging activity, α-glucosidase assay and α-amylase assay	Treatment provides a natural source of anti-oxidants with anti-diabetic and anti-obesity potential	Krishna et al., 2014
Anthelmintic	0.1, 0.3, 1.0 and 3.0 mg/ml	Oil	Fruit	Amplitude, Baseline tension and frequency of spontaneous muscular activity	Helminthic species- *Fasciola gigantica*	Irreversible paralytic effect was observed	Singh et al., 2009
	1:1000 v/v concentration of oil	Oil	Fruit	Amplitude, Baseline tension and frequency of spontaneous muscular activity	Helminthic species- *Ascaris lumbricoides*	Inhibited rhythmic movements of Ascaris (Standard drug- Tetramisole)	Kokate et al., 1980

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	100, 300, 1000 and 3000 µg/ml	Alcohol	Fruit	Motility and Glucose uptake	Helminthic species- *F. gigantica* and *G. explanatum*	Paralyzing effect was observed (Standard drug- Oxyclozanide)	Singh et al., 2007
	100, 300, 1000, and 3000 µg/ml	Alcohol	Fruit	Amplitude, Baseline tension and frequency of spontaneous muscular activity of the amphistome	Helminthic species- *Gigantocotyle explanatum*	Paralytic effect was observed	Singh et al., 2008
	90, 120, 150, 200 mg/ml	Chloroform, Acetone, Ether, Ethanol	Fruit	Larvicidal action	*I. exustsus*	Molluscicides against the snail *I. exustus*	Pandey and Singh 2009
	500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 mg/mL	Methanol, Hexane Fraction, Chloroform fraction	Fruit	Egg hatch assay, Larval motility assay, Larval migration inhibition assay, Adulticidal assay on amphistomes	Adult Amphistomes	Chloroform fraction of *P. longum* possessed maximum broad-spectrum anthelmintic activity comparable to controls (Standard drug- Ivermectin and Thiabendazole)	Koorse et al., 2018
Hepatoprotective	Rats, 50 and 100 mg/kg, p.o.	Ethanol	Fruit	CCl4 induced liver fibrosis	Liver hydroxy proline, serum AST, ALT, ALP, TBL, body weight, liver weight	Anti-fibrotic action was observed due to the flavonoid content of *P. longum*	Christina et al., 2006
	Rats, 50 mg/kg, p.o., 14 days	Ethanol	Fruit		Total protein, Total bilirubin, Total cholesterol, Triglyceride, Urea	By trapping free radical the extract hinders interaction with fatty acid to abolish lipid peroxidation (Standard drug- Silymerine)	Rajeswary et al., 2011
	Rats, 200 mg/kg, 21 days	Aqueous	Fruit	$AlCl_3$ induced hepatotoxicity	ALP, SGOT, SGPT, Creatinine and Bilirubin	Decrease levels of serum biochemical markers	Sharma et al., 2014

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Anti-ulcer	Rats, 50 mg/kg, 60min prior to experiment p.o.	Ethanol	Fruit	Cold restraint stress, Aspirin induced gastric ulcer, Pyloric ligation induced ulcer, Gastric secretion study	Ulcer per stomach, Severity per stomach, Ulcer index, Amount of acid, pepsin, DNA, protein, Total hexose, Hexamine, Fucose, Sialic acid, Total carbohydrate	Balance between aggressive and defensive factors of ulcer production was achieved by treatment	Agrawal et al., 2000
Anti-asthmatic	Rats, 200 mg/kg, p.o.,14 days	Ethanol	Fruit		Mast cell establishing activity (granulated and degranulated mast cell), Mast cell staining, Histopathology	Anti-asthmatic action was noticed (Standard drug-Prednisolone)	Choudhary et al., 2006
Anti-fertility	Female rats, 200 mg/kg from days 1-7 post coital, p.o.	Ethanol	Fruit	Antifertility bioassay method	% Anti-implantation activity	Infertility was observed	Lakshmi et al., 2006
	Rats, 150 and 250 mg/kg, p.o., 30 days	Hexane	Fruit		Estrous cycle, Histological changes in uterus and ovary, Hormone levels in the serum, Anti-oxidant enzymes, NO concentration, measurement of cytokines, -2 expression, antifertility and anti-implantation activity	Treatment decreased FSH, LH, IL-1β, COX-2 expression, activities of SOD, CAT, GPx, GR and GST levels, TNF-α level was increased	Sarwar et al., 2014

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	20 mg/ml	Hexane	Fruit		Hypo-osmotic swelling, Sperm immobilizatio n	Treatment demonstrate contraceptive spermicidal activity *in vitro*	Sarwar et al., 2015
ACE inhibitor	0.5, 1, 1.5, 2, 2.5 mg/ml	Ethyl acetate, Butanol	Fruit		Hippurul-L-histidyl-L-leucine as substrate	Anti-hypertensive action was evaluated	Chaudhary et al., 2012
Mosquito larvicidal	25 mg/L	Methanol	Fruit	Bioassay was performed for Larvicidal Activity for *Aedes aegypti*	Mortality	*P. longum* may serve as replant towards insects	Yang et al., 2002
Anti-platelet	1, 5, 25, 50 g/ml	Aqueous Ethanol, Butanol	Fruit	Rabbit platelet aggregation	Determination of Platelet Aggregation, Measurement of Inositol Phosphates Receptor Binding Assay	Plants contains a constituent that inhibits platelet aggregation as a non-competitive thromboxane A2 receptor antagonist	Iwashita et al., 2007
Anti-amoebic	1000 µg/ml *in vitro*, 900 mg/kg for 5 days *in vivo*, p.o.	Ethanol	Roots	*In vitro* screening, Experimental production of caecal amoebiasis in rats	% caecal amoebiasis of rats cleared and average caecal store in wall and contents in intestine	Treatment possess low *in vitro* activity, exhibited demonstrable *in vivo* activity, against caecal amoebiasis of rats	Ghoshal and Lakshmi, 2002
	1000 µg/ml *in vitro*, 900 mg/kg for Rats, 5 days, p.o.	Ethanol	Fruit	*In vitro* screening, Experimental production of caecal amoebiasis in rats	% caecal amoebiasis of rats cleared and average caecal store in wall and contents	Plant may possess certain compounds other than piperine which are responsible for enhanced efficacy	Ghoshal et al., 1996

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	Mice, 125, 250, 500, 1000 mg/kg, 5 days, p.o.	Ethanol	Fruit	Experimental production of caecal amoebiasis in rats	% caecal amoebiasis of rats cleared and average caecal store in wall and contents	Severity of caecal wall ulceration was reduced in mice which received the extract and metronidazole as compared to the control animals	Sawangjaroen et al., 2004
Anti-stress	Rats, 250mg/kg and 500mg/kg, p.o., 21 days	Aqueous	Fruit	Chronic cold restraint Stress	Plasma levels of glucose, Triglycerides, Cholesterol, Total proteins and Corticosterone	Adaptogenic action was observed	Juvekar et al., 2008
	Rats, 100, 200 and 300 mg/kg, p.o.	Alcoholic	Fruit	Forced swimming stress	Urinary VMA, 5-HIAA, HVA	The levels of VMA and 5HIAA were decreased while HVA levels show increment in mice urine	Kilari et al., 2015
Nootropic	Mice, 250mg/kg and 500mg/kg, 7 days, p.o.	Aqueous	Fruit	Passive avoidance model and Elevated plus maze model	Increased step-down-latency and shortened the transfer latency	*P. longum* may serve as good nootropic agent	Juvekar et al., 2008
	Mice, 100, 200 and 300 mg/kg, p.o.	Alcoholic	Fruit	Scopolamine-induced memory impairment, Y-maze model	Decrease scopolamine-induced memory interruption and increased cognitive performance and working memory in the Y-maze	*P. longum* may serve as good nootropic agent	Kilari et al., 2015

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Anti-convulsant	Mice, 7 days 250mg/kg and 500mg/kg, p.o.	Aqueous	Fruit	Seizures induced by PTZ, strychnine and 4-aminopyridine	Diminution in gamma amino butyric acid levels in brain of extract treated mice, offered protection against PTZ and 4 aminopyridine induced convulsions	Anti-convulsant action validated in this study	Juvekar et al., 2008
Anti-parkinsonian	C57BL/6 mice, 30, 60, and 120 mg/kg for 1 week	Ethanol	Seed	MPTP induced Mouse	Dopaminergic neurons, Tyrosine hydroxylase-immunohistochemistry assay, Antioxidant enzymatic levels	Treatment show ameliorative properties in dopaminergic neurons	Bi et al., 2015
	Mice, 6 weeks, 50 mg/kg, p.o	Ethanol	Fruit	6-OHDA-induced Parkinson's disease	Apomorphine induced rotation, Rotary rod tests, TH, SOD, GSH-Px, GSH, CAT, MDA, NO and NOS level were determined	Treatment improved behavioral abnormality, increase the number of TH cells, up-regulate the activities of SOD, GSH-Px, CAT and GSH, decrease the content of NOS, MDA and NO	Zheng et al., 2014
	Mice,	Ethanol	Fruit	MPTP induced PD	SOD, catalase, LPO, GSH, ATPase, Histopathological examination	Treatment normalized the oxidative biochemical parameters; histopathological examination showed reduction in cell swelling, vascular degeneration and cytoplasmic vacuolation, Decrease level of ATPase indicated the involvement of mitochondria membrane stabilization by treatment	Subburaman et al., 2010

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	Rat, 50 and 75 mg/kg, p.o.	Ethanol	Fruit	Lipopolysaccharide induced dopaminergic neuronal damage rat model	Rotational behavior, Rotarod test, Open-field test, Dopamine, IL-6, IL-1β, TNF-α, ROS and NO activity were determined	Over activated microglial cells were suppressed, IL-6, IL-1β, TNF-α, ROS and NO levels were decreased, Improved behavioral dysfunction increased the levels of DA and DOPAC	He et al., 2016
Anti-cancer	*In vitro*	Hexane, Benzene, Chloroform, Ethyl acetate, Alcohol, Aqueous	Fruit	Prostate (DU 145), Lung (A549), Leukemia (THP-1), ovary (IGR-OVI-1) and breast (MCF - 7) cancer cell line	Cytotoxic assay by sulforhadamine B dye, Cell cycle analysis, Determination of flavonoid and Lipid peroxidation inhibition assay was performed	Cytotoxicity with effect on sub G1 phase	Sharma et al., 2014
	0.1, 0.2, 0.4 mg/ml	Ethanol	Fruit	Malignant melanoma cell line G-361, Human colorectal cancer cell lines HT-29 and HCT116, Ovarian adenocarcinoma cell line OVCAR-3, pancreatic adenocarcinoma cell line BxPC-3, Normal-derived colon mucosa NCM460 cell line	Cell viability, Apoptosis, Binding of annexin V to the externalized phosphatidyl serine, Reactive oxygen species	Treatment leads to selective cell death in leukemia, pancreatic and colon cancer while normal cell were unaffected, Treatment induces caspase-independent apoptosis in cancer cells, without affecting non-cancerous cells, also targeting the mitochondria, leading to dissipation of the mitochondrial membrane potential and increase in ROS production	Ovadje et al., 2014

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	In vitro 500µg/ml and 250 µg/ml, 100 µg/ml, 50 µg/ml Swiss albino mice (10 mg/kg)	Alcohol	Fruit	DLA and EAC cell, L929 cells	*In vitro* cytotoxic activity, solid tumor development, survival of ascites tumor bearing animals	Extract show cytotoxicity, Reduced tumor volume, Increased life span of ascites tumour bearing mice	Sunil and Kuttan, 2004
Ant-hyperlipidemia	Rats, 20 mg/kg	Ethanol	Fruit		Total cholesterol, Triglyceride, HDL, LDL	Treatment normalized lipid markers (Standard drug-Simvastatin)	Jin et al., 2009
	Rats, 100 and 200 mg/kg, p.o.	Oil	Fruit	Streptozotocin -induced diabetic	Glucose level, Insulin level, Liver glycogen content, Glycosylated hemoglobin, Total plasma cholesterol, Triglyceride, and Anti-oxidant parameters	Treatment show increase in body weight, liver glycogen content, plasma insulin, and high-density lipoprotein; and decrease in glycosylated hemoglobin, triglyceride, and total plasma cholesterol (Standard drug-Glibenclamide)	Kumar et al., 2013
Anti-angiogenic	C57BL/6 mice at 10 mg/animal and 10, 5, 1 µg/ml for Primary cultured human endothelial cells	Ethanol	Fruit	B16F-10 melanoma cell-induced capillary formation in mice And VEGF-induced vessel sprouting in rat aortic ring assay	Assessment of tumor-directed capillaries, measurement of IL-1β, IL-6, TNF-α, GM-CSF, VEGF, IL-2, TIMP-1, VEGF-induced proliferation, migration and capillary-like tube formation	Reduced number of tumor-directed capillaries. Increased the levels of IL-1β, IL-6, TNF-α, GM-CSF, VEG, IL-2, TIMP-1, Inhibited VEGF-induced vessel sprouting, proliferation, cell migration and capillary-like tube formation	Sunil and Kuttan, 2006

Abbreviations: Nicotinamide adenine dinucleotide phosphate (NADPH), Glutathione (GSH), Alanine transaminase (ALT), Aspartate aminotransferase (AST), Triglyceride (TG), Cholesterol (CH), Catalase (CAT), Superoxide dismutase (SOD), Adriamycin (ADR), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Total protein content (TPC), Nitric oxide (NO), Cyclooxygenase (COX-2), Vanillyl mandellic acid (VMA), 5-hydroxyindoleacetic acid (5-HIAA), Homo vanillic acid (HVA), Pentylenetetrazol (PTZ), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Tyrosine hydroxylase (TH), 3,4-Methylenedioxyamphetamine (MDA), Nitric oxide synthases (NOS), Glutathione peroxidase (GSH-Px), Dopamine (DA), 3,4-Dihydroxyphenylacetic acid (DOPAC), Dalton's lymphoma ascites (DLA), Ehrlich ascites carcinoma (EAC) cell line, High density lipoproteins (HDL), Low density

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lipoproteins (LDL), Tissue inhibitor of metalloproteinases (TIMP-1), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Minimum inhibitory concentration (MIC), Tumor necrosis factor alpha (TNF-α), Vascular endothelial growth factor (VEGF), Interleukin-1β (IL-1β), Interleukin-2 (IL-2), Interleukin-6 (IL-6).

3.3.1 Anti-inflammatory Analgesic

Many human ailments like asthma, arthritis, atherosclerosis involve the activation of inflammatory response. An inflammatory response originates with the interaction of foreign body matter with macrophage leading to the release of mediators like tumor necrosis factor alpha (TNF-α), which in turn result in the activation of adhesion molecules like intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1) on the endothelial cells surface. This appearance consequently up-regulate the expression of ICAM-1 on white blood cells (WBC) within the lumen of the blood vessel. These activated WBC adheres to endothelial cells and reaches the damaged area resulting in the final activation of T cells. Herbs and spices have illustrated action over the pathway of inflammation and their mediators. Also, small molecules of natural origin have shown to be useful for downregulating the induced cell adhesion molecules (CAM) expression (Cheng et al., 2019; Nonaka et al., 2018). The textbooks for the crude extract of herbs from Indian origin have mentioned the use of *P. longum* fruits as analgesic and anti-inflammatory, specifically used locally for muscular pain (Khare, 2008). In this context, one of the study suggests that the extract of *P. longum* might act through the supraspinal pathway and provides relief in pain (Vedhanayaki et al., 2003), though the dose used in this experiment seems impracticably high without accompanying any toxicological studies. Although the scientific validation for analgesic action of *P. longum* is very scanty providing only the primary data and do not elucidate any signaling pathway for analgesic action. Moreover, 8 species of Piper, along with *P. longum*, were screened for anti-inflammatory action through *in vivo* models (Vaghasiya et al., 2007), and the response was postulated due to cyclooxygenase-2 (COX) suppression or anti-histaminic action without substantiating the mechanism through experimental work. In another study, it was investigated that chloroform extract of *P. longum* inhibited the adhesion of neutrophils to TNF-α stimulated endothelial cells, TNF-α induced expression of nuclear transcription factor-kB (NF-kB), along with the inhibition of ICAM-1, VCAM-1 and E-selectin. Also, *P. longum* seems to interfere with early signaling events in response to stimulus. The extract further repressed microsomal lipid peroxidation, suggesting that the inhibition of CAM and NF-kB may be mediated through inhibition of free radical generation in the form of lipid peroxidation (Singh, N. et al., 2008). This study explained partly

the mechanistic pathways and demonstrated the new therapy for controlling numerous diseases related to increased endothelial leukocyte adhesion molecules. Experiment conducted by utilizing oil of *P. longum* (Kumar et al., 2009), has major lacuna as no authentication of procured plant material is given in the study, dose selection is not defined, and the quality of extracted oil was not assessed. Thus, the study fails to give any valuable scientific update related to the action of fruit oil of *P. longum*. Kumari et al., has further compared the anti-inflammatory activity of two varieties of *P. longum* fruit by carrageenan and formaldehyde-induced paw edema. Carrageenan induced paw edema model is for acute inflammation with consequent release of histamine, 5-HT and prostaglandins, while formaldehyde induced paw edema model measures proliferative action during inflammation (Kumari et al., 2012). However, this experiment did not elucidate differences in phyto constituents of varieties of *P. longum* and conclusions are drawn only on the basis of two animal models with a single dose only.

Granting the mentioned traditional use of *P. longum* as anti-inflammatory and analgesic, systemic studies including *in vitro* and *in vivo* experiments elucidating the exact mechanistic basis are lacking. Also, no proper distinguishing actions are reported over the pro-inflammatory mediators and their molecular signaling cascade. Piperine, being one of the potent agonist of human vanilloid receptors (McNamara et al., 2005), still, no relation has been identified between the actions of extract of *P. longum* and its bio-active principle over vanilloid receptors. Thus, in-depth analysis is required for illustrating the signaling cascade in case of anti-inflammatory and analgesic actions of the plant and its metabolites.

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3.3.2 Anti-oxidant

Anti-oxidant agents are those which decline the oxidative stress and slackens the frequency of pathological circumstances instigated by oxidants. Oxidative stress is harmful to the cellular organizations as they cause peroxidation of lipid membrane consequent damage to membrane integrity and cell death, denaturation of proteins comprising enzymes, ion channels and strand breakage in DNA. These cellular changes clearly indicate that escalation of oxidants in the body leads to aliment like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. The occurrence of phenols, flavonoids, and terpenes in plants make them better anti-oxidant agents, which are not only economic but also diverse and produces action without side effects (Barua et al., 2014; Nile et al., 2018). On the contrary, before translating the health benefits of anti-

oxidants for biomedical sciences, detail analysis of plant will be required, as they might also lead to toxicity due to pro-oxidative activities (Yang et al., 2018). The action of *P. longum* extract have been confirmed using isoproterenol induced oxidative stress (Jagdale et al., 2009), adriamycin induced oxidative stress (Wakade et al., 2008), and in monosodium glutamate induced stress (Thomas et al., 2009). *P. longum* extract also produces synergistic anti-oxidant action when administered with other herbs (Samudram et al., 2009). The above-reported experiments have confirmed the use of *P. longum* in pathology associated with oxidative stress, although limited information on dose-dependent effect is available. Moreover, the anti-oxidant potential of the extract is postulated to be due to the presence of phenolic compounds and flavonoids. Also, the action has been evaluated through various chemical assays like DPPH and free radical scavenging assays, which do not provide any pharmacological relevance and limits the validation of established biological action. Scientists can further adopt chromatographic techniques, apply some *in vitro* biological test(s) exploiting cell lines and simulated digestion and *in vivo* assessments, which will reveal the dose and time-dependent action of *P. longum* extract and can be utilized for the development of pharmaceutical and/or nutraceutical products. Additionally, protection offered against oxidative injury paves path to employ this plant as chemopreventive and in neurodegenerative disorders, which is further detailed in below-mentioned sections.

3.3.3 Anti-microbial

Modern day bacterial infections are treated by the use antibiotics. Although by various mechanisms, bacteria develop resistance to these antibiotics. New mechanisms are now being elucidated for resistance, which are spreading all over the world and making the infections harder to treat. It has been observed that plants naturally have resistance against bacterial pathogens (González-Lamothe et al., 2009). The resistance for bacterial pathogen in plants develop at different levels and through different mechanisms, among which synthesis of anti-bacterial agents is one of them. For human betterment, these anti-microbial agents can be utilized either in isolated form or as a whole extract (Gonelimali et al., 2018; Srinivasa Reddy et al., 2001). Thus, various studies have been conducted to seek for anti-microbial property in *P. longum*. Arambewela and co-workers first reported the anti-microbial action of volatile oil of *P. longum* seeds (Arambewela, 1999). Khan and Siddiqui tested extracts of *P. longum* against

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Staphylococcus albus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Bacillus megaterium and Aspergillus niger. The effective action of *P. longum* extract against these bacterial strains was stipulated due to the alkaloids and terpenoids present in crude drug (Khan and Siddiqui, 2007). *P. longum* also seem to produce action against bacterial strains like *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus flavus, Bacillus cerus, Bacillus subtilis, Klebsiella aerogenes, Klebsiella pneumonia, Citrobacter freundii, Pseudomonas aeruginosa, Staphylococcus typhrium, Proteus mirabilis, Proteus vulgaris* (Vaghasiya et al., 2007). However, the above-mentioned experiments did not represent any dose dependent action, as the studies were performed with single dose, with no information over mechanism of action. Ali et al., has performed antibacterial and antifungal activity of *P. longum* extract in detail against 13 pathogenic bacteria (5 gram-positive and 8 gram negative) and 6 fungi. It was observed that the extracts of various solvents were sensitive towards specific bacterial strains and the action were less prominent towards fungal strains (Ali et al., 2007), though the doses employed were on the higher side. Aneja and co-workers has investigated the activity of *P. longum* against selected bacterial and oral fungal pathogens i.e. *Streptococcus mutans, Staphylococcus aureus, Candida albicans and Saccharomyces cerevisiae*. The action by *P. longum* was more prominent to treat oral fungal infection especially against *C. albicans* (Aneja et al., 2010).

Cooperatively, it can be stipulated that essential oil and alkaloid content of *P. longum* is responsible for their anti-bacterial property. However, the above-reported studies have only used conventional methods like agar well/disk diffusion methods, thus advancements in methodology need to be adopted to validate the anti-microbial action. Methods such as bioautography, flow cytofluorometric, bioluminescence assay can be adopted to test the anti-microbial susceptibility of the extract. In-depth experiments need to be conducted over the toxicity, mechanism of extract against microbes and whether the extract penetrates the microbial body and interact with the transporters. Nevertheless, a time-dependent and concentration-dependent anti-microbial effect of *P. longum* extract need to be evaluated.

3.3.4 Anti-cancer

Due to the presence of multiple phyto-constituents in herbs, they provide protection against cancer through various mechanism. Some of them involve (a) reducing oxidative stress, (b)

reducing inflammation and depression in inflammation-associated pathways, (c) influencing phase I and II metabolism enzymes, and (d) anti-tumorigenic mechanism like modulation of transcription factors, antiapoptotic proteins, proapoptotic proteins, protein kinases, cell cycle proteins, cell adhesion molecules and growth signaling pathways. Today, one of the important class for anticancer drugs like vincristine, vinblastine, taxol, topotecan are derived from herbal sources (Kaefer and Milner, 2008; Lin et al., 2017). Therefore, there is continued quest for development of new and potent molecules against cancer which are derived from natural sources. Sunila and Kuttan studied the anti-tumor effect of *P. longum* extract, evaluated through *in vitro* and *in vivo* studies (Sunila and Kuttan, 2004), although this study did not incorporate any standard agent for comparison. Additionally, there was no validated method adopted to study the cellular/molecular changes in cancer cell lines. In another study, hexane extract of *P. longum* extract was most effective against all cell line with 90% cytotoxicity and benzene extract demonstrated 63% cell cycle inhibitory action at subG1 phase (Sharma et al., 2014). This study further does not give dose response relation, with no validation of molecular pathway. The results from Ovadje and co-worker demonstrate that extract of *P. longum* produces selective apoptotic cell death of malignant cells by directing the changes in mitochondrial membrane potential, following a nongenomic approach (Ovadje et al., 2014). Owning the ethnopharmacological relevance of *P. longum* as anti-cancer herb, the above-reported studies have been able to successful elucidate the actions of *P. longum* extract against various cancer cell lines as well as its action over normal cells. It has revealed the effectiveness in very less concentration indicating the synergistic action of bio-active moieties present in the extract of *P. longum*. The phytochemical identification presented in the studies have further aided in relating the potential of extract with its isolated moieties. However, question can be still raised about the comparative efficiencies of fruit, root and stem of *P. longum* as anti-cancer herb.

The above-mentioned studies not only prove selective cytotoxicity of *P. longum* extract towards cancer cells but also provide evidence for anti-cancer action, as the experiments performed by Ovadje et al., 2014, and Sunila and Kuttan, 2004, have reported the reduction of tumor volume, increase life span of ascites tumor bearing mice and arresting the growth of colon cancer tumors in immunocompromised mice upon treatment with *P. longum* extract. However, further experiments can be conducted to validate the efficiency of extract via targeting the genomic and autophagy pathways.

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3.3.5 Anti-parkinsonian

Parkinson's disease (PD) is progressive neurodegenerative movement disorder. The loss of dopaminergic neurons in the substantia nigra pars compacta (SN-pc) is considered a major underlying pathological event. This further results in motor disturbances and formation of Lewy bodies pathology. The currently available therapies for PD involves restoration of dopamine levels by levodopa, direct dopaminergic agonists or drugs reducing the metabolism of dopamine referred as MAO-B inhibitors (Jin et al., 2019). However, an important pathological feature of PD i.e. disturbance in mitochondrial function and increased oxidative stress have not received strong molecules to target. Herbal medicines like *Bacopa monnieri, Mucuna pruriens, Withania somnifera, Curcuma longa, Gingko Biloba, and Camellia sinensis* have shown encouraging effects towards this pathway (Srivastav et al., 2017). *P. longum* extract show protection against MPTP induced changes in mice model which is based on the oxidative damage and free radical generated pathological changes in PD (Subburaman et al., 2010). Although the experiment has major flaws as the study was conducted with single dose only and no information regarding the extraction procedure, solvent and part of plant was indicated. Also, this report did not consider the behavioral evaluation. The efficacy of this plant to be used in PD was also supported by Bi et al., using MPTP model. The study revealed that the anti-oxidant levels were brought to normal values at the dose of 60 mg/kg of *P. longum* and significantly amplified the levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in mice brain (Bi et al., 2015), however, the part of *P. longum* used in the study remains unclear. The preventive action of *P. longum* was also studied in 6-hydroxydopamine (6-OHDA) induced PD rat model. 6-OHDA, being structural analogue of catecholamines, produces its neurotoxic effect by accumulation into dopaminergic neurons, subsequently leading to heavy neuronal damage and is employed to study biochemical alterations in PD (Song et al., 2016; Zheng et al., 2014). To further validate the underlying mechanisms, He et al. have investigated the protective effect of *P. longum* for dopaminergic neurons against inflammation-mediated damage. This model has been put forward due to the fact that neuroinflammation may be involved in the death of dopaminergic neurons consequently leading to activation of microglia cells. *P. longum* being able to provide protection in this model provides evidence that it has protective effects on dopaminergic neurons against inflammatory

reaction induced damage (He et al., 2016; Sharma and Nehru, 2015). In all the above-mentioned reports, anti-inflammatory and the ability of *P. longum* to prevent oxidative injury was considered for treating PD and the preventive mechanisms have been well elucidated. Collectively, these studies suggest that *P. longum* might serve as multi-component and multi-targeted approach for the treatment of PD.

3.3.6 Anti-stress

Panax ginseng, Rhodiola rosea, Withania somnifera, and Bryonia alba are some of the well-established adaptogens. The presences of phenolic compounds, tetracyclic triterpenes and fatty acid in plants make them appropriate as adaptogens. Adaptogens sever as tonics and are indicated in stress, neurological and psychological disorders. Pharmacologic assessment of adaptogens involves exposure to altered environmental surroundings, radiation, toxic substances, starvation, fear, and chronic diseases. Also, the most important feature of adaptogens is the ability to increase resistance to both physical and emotional stress (Özdemir et al., 2018; Wal et al., 2019). *P. longum* has also been evaluated for combating stress. Stress production in body leads to alteration of normal physiology and is involved in various disease like diabetes mellitus, hypertension, depression, anxiety, immunosuppressant etc. Stress also leads to the formation of free radicals and produce damage to neuronal cells. Mainly, the plants having adaptogenic action are suggested to provide relief under stressed conditions (Gulati et al., 2016). Kilar and co-workers have developed a model for evaluating anti-stress action of *P. longum* by non-invasive assessment of vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) as the biomarkers for neuronal damage in stressed mice, produced by forced swimming. The levels of biogenic amines was not changed during normal condition but previous administration of extract blocked the stress induced deviations in a dose-dependent manner (Kilari et al., 2015). Juvekar et al., has also postulated that anti-stress activity of *P. longum* is attributable to combating the stimulation of hypothalamus pituitary adrenal (HPA) axis in stressful condition (Juvekar et al., 2008). The major lacuna in both the studies lies on evaluation in a single animal model, lack of validation for evaluating stress response in multiple models for reproducible results. Most of the biochemical parameters being evaluated were non-specific to stress and have been suggested to change due to multiple factors, except cortisol and therefore the studies did not support the involvement of HPA axis. Further, both the studies did

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not confirm the part of the plant being used in experiment. Thus, there is need for better pharmacological assessment of *P. longum* extract to evaluate the adaptogenic action.

3.3.7 Nootropic

Nootropic is a term utilized for therapeutic medications or supplements that positively affect cerebrum function. Various pharmaceutical medicines are in the market, which has been utilized for their neuroprotective property by adjusting the parity of specific cerebrum working. A portion of the medications follow up on the improvement of cerebral blood stream, metabolic rate of cerebral oxygen use and metabolic rate of cerebral glucose in perpetual impeded human cerebrum work, i.e., stroke, poor cerebrum blood stream, dementia and pseudo dementia. A few prescriptions are acquired from the therapeutic herbs which have demonstrated memory upgrading properties by ideals of their proactive phytochemical constituents (Kumar et al., 2015; Srivastava et al., 2019). Thus, in persistence of the anti-stress activity, Kilari et al., has also provided evidence in support of nootropic activity of the *P. longum* extract. As it has been anticipated that stress deflates memory, thus, the extract was evaluated for scopolamine-induced memory impairment in Y maze. It was observed that extract of *P. longum* prominently differentiated arm entries, duration of time spent in the novel arm, transfer latency for novel arm and spontaneous alteration behavior, compared to scopolamine treated groups (Kilari et al., 2015). Similarly, Juvekar and co-workers have also confirmed the nootropic action of *P. longum* extract. These studies self-postulated that anti-cholinesterase and anti-oxidant action of *P. longum* proclaim its application in cognition health (Juvekar et al., 2008). Both the studies did not confirm the part of *P. longum* for preparation of extract and did not evaluate any biochemical parameters. Besides, the conclusion is drawn on the basis of behavioral parameters, which do not effusively illustrate the protective action of extract.

The old Indian text of ‘Charka Samhita’ has also mentioned the role of *P. longum* fruit to cure dementia and improve cognitive functions (Manyam, 1999). SuHeXiang Wan is a Chinese formulation, which is mentioned for the treatment of seizures and cognition improvement, constitutes *P. longum* as one of the ingredient. Further, *in vitro* action of *P. longum* have been reported to increase cell viability in the presence of amyloid beta (Jeon et al., 2011). Above evidences indicate a tremendous scope for further experimental work, which needs to be executed to understand the actions of *P. longum* in cognitive improvement and to determine

whether the same can be exploited to cure Alzheimer's disease, by emphasizing over the cerebral functions, elucidating signaling pathways, the involvement of neurotransmitters and receptors for prevention of dementia.

3.3.8 Anti-epileptic

The Ayurvedic and Chinese literature has provided reports for utilization of herbal medicine in treatment of epilepsy, for instance, daily fresh juice of *Centella asiatica* with honey, garlic juice in oil, and powdered root of wild *Asparagus racemosus* with milk. The scientifically validated herbal extract seems to produce action by prolonging the latency to seizures or decreasing the seizure duration or seizure related mortality. Also, molecular targets which are associated with the recovery of epilepsy through herbal treatment involves interaction with $GABA_A$ receptors, modulation of chloride channels, Ca^{2+} channels, voltage-gated K^+ channels, 5-HT3A receptor channels, hyperpolarization-activated cyclic nucleotide-gated channels, mitogen-activated protein kinase and transient receptor potential channels. *P. longum* containing formulation has also been utilized for treatment of insomnia and epilepsy, however, scientific data is scanty in this context (Schachter, 2009; Sucher and Carles, 2015). Experiments of Juvekar et al., revealed that the extract offered protection against PTZ-induced convulsions but did not show any protection against strychnine and 4-aminopyridine-induced convulsions in mice. The involvement of GABAergic system was postulated in providing protection in the animal model as the level of GABA was significantly decreased in extract treated group as compared with control mice brain (Juvekar et al., 2008). The study has given a basic understanding about the involvement of GABAergic system during treatment with *P. longum* extract, however, a detail *in vitro* and *in vivo* studies are required to understand the interaction of GABA receptor, other channels and neurotransmitters upon *P. longum* treatment. There are only few studies are available and the potential has only been shown in acute seizures and not validated in chronic seizure models like kindling. In view of the high incidence of pharmacoresistance in epilepsy despite introduction of third generation anti-epileptic drugs, there is an urgent need to investigate novel drugs to combat the same without adverse effects on cognitive functions. Further, being recognized as sedative, a therapeutic window to minimize the toxic effects, along with strong evidence for penetration of extract across blood brain barrier, needs to be established.

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3.3.9 Anti-hyperglycemic

The utilization of herbal medicine to combat diabetes is due to the presence of enormous phytoactive constituents in plants which produce their action via either release of insulin from pancreatic β-cells, repressed glucose absorption in gut, stimulated glycogenesis in liver or increased glucose utilization by the body. Therefore, herbal medicines may serve as an alternative therapy for insulin (Joseph and Jini, 2011). *P. longum* extract has demonstrated activity as α-glucosidase-I inhibitor action (Pullela et al., 2006), in alloxan-induced diabetic model (Manoharan et al., 2007) and in streptozotocin induced diabetic rats (Nabi et al., 2013). The experiment conducted by Nabi et al., holds advantage in experimental design, since well formulated short term and dose-dependent studies were conducted to screen out the efficacy of *P. longum* extract. Experiments of Manoharan and co-workers extrapolated the role of *P. longum* during diabetic condition by stimulating the activity of the liver to maintain the normal homeostasis of blood glucose, activating insulin secretion from surviving pancreatic β-cells and avoiding the glycosylation process to improve the glycemic control mechanisms (Manoharan et al., 2007). Further, experiments have confirmed that inhibition of α-glucosidase and acarbose enzyme by *P. longum* extract seems to the foremost fundamental mechanism for anti-diabetic property. *P. longum* extract being able to produce significant changes in these markers might help in combating diabetic symptoms and also associated comorbidities like diabetic retinopathy (Kumar et al., 2013). Ayurveda Pharmacopeia has mentioned the use of *P. longum* for diabetes, also these experiments will serve as strong pre-clinical evidence for utilization of this plant in treatment of diabetes, as well as in associated comorbidities.

3.3.10 Hepatoprotective

Chronic liver damage subsequent to liver repair leads to liver fibrosis. This stage of liver occurs when healthy tissues of liver becomes scarred. Liver fibrosis is the exorbitant gathering of extracellular matrix proteins incorporating collagen that happens in many sorts of continual liver illnesses. Advanced liver fibrosis results in cirrhosis, liver failure, and hypertension and may require liver transplantation. Being a severe threat to humans, anti-fibrotic agents are still in developmental phase. Thus, hepatoprotective herbs may be useful in such clinical condition (Latief and Ahmad, 2018; Wang et al., 2016). Subsequently, fibrosis is portrayed by unreasonable scarring, over the top creation of collagen due to diminished collagenolytic

movement. This collagen is balanced out by its triple helix structure and this structure is given by an amino acid, hydroxyproline. Henceforth, there is adjustment in extracellular matrix (ECM), which is a noteworthy maker of fibrotic neomatrix. The changed ECM is corrupted by MMPs (Matrix metalloproteinases), also the MMPs are in sequence hindered by TIMPS (Tissue inhibitors of matrix metallic proteinase). Subsequently, articulation of TIMP is expanded in human and rodent to demonstrate fibrotic liver. In human liver, the level of TIMP articulation associates with the degree of fibrosis is evaluated by hydroxyproline. So estimation of hydroxyproline content is a magnificent biochemical marker to evaluate the level of fibrosis (Campana and Iredale, 2017). The protective action of *P. longum* has been demonstrated in CCl_4 induced liver fibrosis model (Christina et al., 2006). CCl_4 has been found to induce hepatic toxicity by metabolic activation. It is being converted to $CCl_3^{\cdot}$ free radical by cytochrome P450 in endoplasmic reticulum. This free radical generation causes lipid peroxidation, demolition of Ca^{2+} homeostasis and cell death. Thus, it selectively serves as good model to study liver toxicity (Li et al., 2019). The *in vivo* hepatoprotective action of *P. longum* has also been validated by producing $AlCl_3$ induced liver toxicity. This metal induced toxicity was reversed by *P. longum* extract and the action was ought to be due to anti-oxidant effect (Sharma et al., 2014). The flavonoids present in the *P. longum* seems to be responsible for hepatoprotective action, although some studies have reported the action with single dose and in acute regimen, which was amended in other report.

3.3.11 Anti-hyperlipidemic

Ischemic heart disease is one of the foremost reasons of mortality around the domain, inappropriate lifestyle, nutritional modification and hyperlipidemia are among the major risk factors associated with this disease. The currently used therapy for hyperlipidemia involves the use of statins, which produces severe side effects like myopathy and memory loss. Thus, there is continued quest to develop new molecules which show prominent action of lipid levels with no side effects (Taghizadeh et al., 2019). The potential herbs with hypolipidemic action have common target of lowering the serum lipid levels, although physiologically, herbs like *Ocimum basilicum* produces changes in lipid oxidation, *Vaccinium angustifolium* induce inhibited lipid accumulation, *Taraxacum officinale* inhibit adipocyte differentiation and lipogenesis, and *Nigella sativa* decreases hepatic β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase

activity (Rouhi-Boroujeni et al., 2015). Experiments conducted to validate the action of *P. longum* involves assessment of lipid markers in blood suggesting its beneficial effect in case of hyperlipidemia (Kumar et al., 2015; Kumar et al., 2013). However, these experiments seem preliminary requiring dose optimization and mechanistic approach for validating its hypolipidemia action.

3.3.12 Anti-platelet

Numerous studies are now being conducted to screen out natural products which improves the circulation and maintain hemostasis. Natural products seems to interfere with the primary hemostatic system that regulates receptors such as catecholamine, collagen, thrombin, prostacyclin, adenosine diphosphate (ADP), prostaglandins D2 (PGD2), prostaglandins E2 (PGE2), Thromboxane A2 (TXA2), cyclic AMP (cAMP), cyclic GMP (cGMP), and calcium have been recognized to be the operative objectives of anti-platelet treatment (Nor et al., 2016). Only one study has been reported which evaluated the inhibitory action of *P. longum* extract on platelet. U46619-induced platelet aggregation model was used for *in vitro* evaluation of inhibitory action on rabbit platelet aggregation, which was strongly inhibited by ethanol extract of *P. longum*. To further clarify the mechanism of inhibitory action, the extract was additionally evaluated in thrombin, ADP and 5-HT induced platelet aggregation. It was found that most potential inhibitory action was observed in U46619-induced platelet aggregation model which is a TXA_2 receptor agonist. *P. longum* treatment also inhibited the U46619-induced phosphoinositide hydrolysis, which was further established by performing receptor binding assay of TXA2 antagonist, [3H]SQ29548, to TXA2 receptor. The extract seems to inhibit [3H]SQ29548 binding to TXA2 receptor at an IC_{50} value of 75 µg/ml. Overall, these results concluded that the extract from *P. longum* produce its inhibitory effect on platelet aggregation as a TXA2 receptor antagonist (Iwashita et al., 2007). The study holds advantage in terms of involving various solvents to prepare extracts of *P. longum* and has demonstrated concentration-dependent response, although part of plant for the extract preparation was not mentioned. Moreover, improvements to understand the action of extract in a more efficient way can be made by involving negative control and standard agent in study design, along with determining *in vivo* bleeding time, *ex vivo* bleeding time and ruling out the possibility of anti-thrombotic effect of *P. longum*.

3.3.13 Anti-angiogenic

Angiogenesis is the process of formation new blood vessels and is indicated in tumor metastatic. Anti-cancer treatment is gaining intersect for angiogenic therapy due to low toxicity, extensive effectiveness, and the target that the neovasculature endothelial cells are genetically stable and implausible to advance acquired resistance. Multiple studies have recommended the inhibitory action of natural product and dietary compounds on tumor specific angiogenesis (Dai et al., 2017; Huang et al., 2015; Ye et al., 2015). The study conducted by Sunil and Kuttan, suggest that the anti-angiogenic action of *P. longum* is associated to the regulation of proinflammatory cytokines growth factors in angiogenesis induced mice, along with inhibitory action on proliferation, migration and differentiation of endothelial cells (Sunila and Kuttan, 2006). Thus, it can be concluded that *P. longum* has novel molecular mechanism that restrict the mutual angiogenic signaling pathways, however, the animal experiments did not establish dose-effect relationship.

3.3.14 Immunomodulatory

Immunotherapy is characterized as the way to deal with malignant growth by producing or enlarging a safe reaction against it. Immunomodulators are holding prominent promise for their utility in clinical aspects, as they can be used with chemotherapy and may provide additional benefits (Khalil et al., 2016). Moreover, chemotherapy being commonly practiced, yet produces severe side effects like immune suppression, bone-marrow suppression, organ damage etc. Moreover, immuno-modulators of herbal origin such as *Trametes versicolor*, *Astragalus membranaceus*, *Panax ginseng* seems to exert fortification against such side effects (Yarnell and Zimmerman, 2017). The immuno-modulatory action of alcohol extract of *P. longum* when evaluated in mice, resulted in increment of the leukocyte count, bone marrow cellularity, α-esterase positive cells and total antibody production. These results suggest that immunomodulatory activity of *P. longum* may be due to the combined action of humoral and cell-mediated immune responses (Sunila and Kuttan, 2004). Besides the absences of standard comparison group in the study design, these experiments may be taken forward as they indicate the action of *P. longum* over stem cell proliferation and hemopoietic system on chronic treatment.

3.3.15 Angiotensin converting enzyme (ACE) inhibitor

ACE is responsible for converting angiotensin I to angiotensin II, which is a potent vasoconstrictor. ACE inhibitors lessen the movement of the renin-angiotensin-aldosterone framework (RAAS) as the essential etiologic occasion in the advancement of hypertension in individuals with diabetes mellitus, as a feature of the insulin-obstruction disorder or as a sign of renal illness (Cabandugama et al., 2017). Herbs have been reported to produce vasodilation from increasing level of nitric oxide (NO), prostaglandin I2 (PGI2), blockage of Ca^{2+} channel, opening of K-ATP channel, inhibition of ACE and reduction of endothelin-1, thus sinking the risk of cardiovascular diseases (Chrysant and Chrysant, 2017). *P. longum*, being traditionally recommended for cardiovascular disease, was evaluated for ACE inhibitory activity. The experiment indicated IC_{50} value of 1.40 mg/ml through *in vitro* ACE inhibition assay, using hippuryl-L-histidyl-L-leucine as substrate (Chaudhary et al., 2013). However, this experiment had some shortcomings, including no standard drug for comparison and evaluation of only single parameter, resulting in lower reliability and insufficient details to draw conclusion. Thus, an experimental animal model must be established to further verify the effects of *P. longum* on the cardiovascular system.

3.3.16 Anti-arthritis

Rheumatoid arthritis (RA) is chronic auto-immune disorder that mainly affects joints and results in painful and swollen synovial tissue. The pathogenesis of RA includes synovial cell multiplication, angiogenesis, pannus development and cell insusceptible actuation by T cells, B cells and macrophages, which consequently leads to deformation in ligament and bone disintegration. The aim of the treatment is to suppress the pain and inflammation. The available medication helps in relief of symptoms by using NSAIDs while the disease progression is reduced by disease-modifying anti-rheumatic drugs although these medications in-turn produces severe side effects leading to complications. This requires the improvement in RA treatment with satisfaction of security and adequacy to combat against neglected restorative need (McInnes and Schett, 2011; Rahman et al., 2017). Owning to the strong anti-inflammatory actions of *P. longum*, and mentioned use against RA in Ayurvedic Pharmacopeia of India, this herb has also been evaluated against RA. Paw volume displacement, as measured on 4^{th}, 8^{th}, 14^{th} and 21^{st} day of experiment, represented maximum percentage inhibition by 46.23% at the dose of 400 mg/kg

of *P. longum* (Yende et al., 2010). Nonetheless, lack of biochemical evaluation and use of limited dose presents the inadequate data to analyze the effectiveness of the extract. Also, in spite of chronic administration of extract to animals, toxicological studies were not conducted. However, in light of the fact that the variables influencing RA are copious, the exact mechanism of *P. longum* in the treatment of RA requires progressively fundamental research and further inside and out research including scanning for variables that influence the RA and investigating the related signaling molecules for anti-arthritis effects.

3.3.17 Anti-ulcer

Gastric ulcers are creating major economic burden as they are often associated with smokers, patients on chronic therapy of NSAIDs or the person with chronic alcoholism. One of the close relative from Piper genus, *P. betel*, show prominent protective action for ulcer including other herbs like *Myristica malabarica, Teucrium polium, Phyllanthus emblica, Centella asiatica* etc. (Pradhan et al., 2013). The findings of Agrawal et al., has suggested that *P. longum* extract significantly balanced the offensive actions of ulcer although no prominent action was observed on gastric mucosal glycoproteins and mucin secretion (Agrawal et al., 2000). Moreover, 10 days of administration of *P. longum* extract to foot shock induced stress animals also provided 90% protection in ulcer severity as compared with doxycycline (Yadav et al., 2015). The studies lack the examination of PG release, mucosal blood flow, cell proliferation and evaluation based on biochemical indicators.

3.3.18 Anti-asthmatic

National asthma campaign has reported that 60% of case of moderate asthma and 70% cases with severe asthma rely on alternative medical therapy. Also, the classification of anti-asthmatic drug is derived from natural sources, thus herbal medicine intervention can be considered as a good alternative for asthma. The herbal extracts which show prominent action in this chronic inflammatory disorder include *Picrorrhiza kurroa, Boswellia serrate, Solanum xanthocarpum, Tylphora indica* etc. (Huntley and Ernst, 2000). The use of *P. longum* for bronchitis and asthma has been mentioned in Ayurvedic formulation, although the scientific validation is still required. In one experiment, it was postulated that the extract is active against type I allergic disorder due to its ability to prevent the release of allergic mediators from mast cells (Choudhary, 2006). In

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another study, *P. longum* fruit extract at the dose of 200 mg/kg, show bronchorelaxation with 83% preventive action in histamine-induced bronchospasm model of Guinea pig. Also, the neurotransmitter imbalance of dopamine leads to higher levels of histamine in haloperidol-induced catalepsy in mice which was normalized with the extract. Taking an account over the anti-inflammatory action of *P. longum* investigation of passive paw anaphylaxis in rats, a dose of 200 mg/kg demonstrated significant inhibition in paw edema volume when compared with standard drug dexamethasone. All these parameters has supported that the petroleum ether extract of *P. longum* possess substantial anti-asthmatic activity (Kaushik et al., 2012). Owing to traditional citation of *P. longum* for respiratory disorders, the above experiments only provide superficial evidence with no indication of signaling pathways and fails to provide action of *P. longum* on specific respiratory organs. Also, the anti-histaminic and anti-inflammatory action of *P. longum* have been postulated for preventive action towards asthma, with no biochemical validation, thereby generating ambiguity in authenticating of this action.

3.3.19 Anthelmintic

Parasitic worms are referred to as helminths, which belongs to the class of flatworm (platyhelminthes) and nematodes (roundworms). The parasitic infection caused by these flukes and worms have become a major health problem throughout the world. These parasites cause haemorrhage and connective tissue expansion at the site of connection, vacuolar degeneration in liver and hyperplasia in bile channel, accordingly, truly influencing wellbeing and efficiency of contaminated creatures. These disorders are only effective towards chemotherapy which reversely causes adverse reaction, thus plant based therapy are now being developed as the choice of treatment for such parasitic infections (Valentynivna, 2017). The use of *P. longum* as anthelmintic has been reported in Ayurvedic Pharmacopeia, nevertheless, the first scientific validation came from the report of D. Cruz et al., which evaluated the action of essential oil of *P. longum* against *Ascaris lumbricoides* which is a large roundworm of human (D'Cruz et al., 1980). This study was supported by Kokate et al. who showed paralysis of *A. lumbricoides* within 15 minutes of treatment of *P. longum* oil (Kokate et al., 1980), however dose range was not mentioned in this study. *Fasciola gigantica* and *Gigantocotyle explanatum* are the two parasitic flatworm belongs to the trematoda class and phylum platyhelminths. The growth of these flatworms depends on the primary source of energy that is glucose, and the inhibition of

this bio-energy system results in deactivation of parasite (Ullah et al., 2017). The extracts of *P. longum* reduced mass motility of both the flukes resulting in paralyzing action, due to inhibition of glucose uptake and was more prominently observed in *G. explanatum* (Singh et al., 2007), moreover, paralysis of *G. explanatum* amphistome was also observed by progressive decline in the spontaneous muscular activity upon treatment with extract (Singh, T. et al., 2008; Singh et al., 2009). They further studied the action of *P. longum* on *Fasciola gigantica* whole fluke and strip preparation. The changes in spontaneous muscular activity by fruit oil were suggested to occur due to the involvement of neuromuscular system, thus, *P. longum* exhibited a potential to become newer anthelminthic herbal based formulation (Singh et al., 2009). However, Koorse et al., 2018; has demonstrated the *in vitro* ovicidal, larvicidal and adulticidal action of fruits of *P. longum*, methanolic extract was extremely active against ova by IC_{50} of 0.026 mg/mL, n-hexane portion was strong in persuading larval mortality with IC_{50} of 1.383 mg/mL while chloroform fraction repressed larval migration with IC_{50} of 1.796 mg/mL. These results provide evidence in support of anthelmintic action of *P. longum* (Koorse et al., 2018). Thus, *P. longum* provides multi target approach for treatment of helminthic diseases and can provide a better formulation as anthelminthic. Nevertheless, efficient *in vivo* models need to be established for evaluation of the efficiency and toxicity of *P. longum*.

3.3.20 Anti-amebic

Infection of human gastrointestinal system by *Entamoeba histolytica* is termed as amebiasis. *E. histolytica* is a protozoan parasite which attacks the intestine mucosa and spreads to vital organs in body. The infection spreads mainly due to fecal-oral transmission of protozoa and causes deaths worldwide. The currently available treatment causes side effects, thus, to combat such effects, there is continued research on natural products which produces its action for longer duration (Nakada-Tsukui and Nozaki, 2016). Ghosal et al., has evaluated the action of *P. longum* fruit and root, considering that it has been traditionally used for intestinal disorders (Ghoshal and Lakshmi, 2002; Ghoshal et al., 1996). However, their experiments failed to provide dose-dependent response. Similar *in vivo* data has also been reported by Sawangjaroen et al., using methanol extract of *P. longum* fruit at the doses of 125, 250, 500 and 1000 mg/kg for 5 days, which show 0, 46, 93 and 100% cure respectively, on caecal amoebiasis in mice (Sawangjaroen et al., 2004). However, the common limitation observed in the experiments was that the dose of

P. longum was too high in these studies, which will not deliver reliable experimental data for clinical applications. Besides, it is apparent that *P. longum* possesses high efficiency for treatment of amoebiasis, further emphasis on its mechanism necessitates on exploring the utilization of oxygen, which could be important for the survival of the parasite in the host and may afford targets for therapeutic attack.

3.3.21 Anti-fungal

Dermatophytosis, commonly termed ringworms, is caused by fungal infection refereed as dermatophytes. Dermatophytes are the group of closely related fungi which have the capacity to invade keratinized tissue like skin, hair and nails. These infections are cutaneous and do not invade soft tissue, however, the infections are posing major health issue in the world. These species producing cutaneous infections are also referred as keratinophilic mycoflora. As the available anti-fungal drugs are producing serious side effects, thus, there is need for alternative therapy (Martinez-Rossi et al., 2017). Das et al., has performed sequential extraction of leaves of *P. longum* wherein chloroform and methanol extract were effective against *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, Microsporum fulvum* and *M. gypseum,* serving as a compelling phytoremedy against dermatophytes (Das et al., 2012). Although this experiment failed to show concentration-dependent response. Prassanna et al., has used methanol root extract of *P. longum* against *Chrysosporium anum, C. lobatum, C. tropicum, Microsporum gypseum, M. nanum, Trichophyton ajelloi, T. mentagrophytes, T. terrestre* at the concentration of 250, 500 and 1000 µg using agar well diffusion method (Prassanna et al., 2011). These studies postulate that *P. longum* may be exploited against keratinophilic species and also combat against the multidrug resistance which makes fungal infections difficult to treat. Furthermore, *in vivo* efficacy and safety to determine the viable prospects of the extracts as anti-dermatophytic agents need to be highlighted.

3.3.22 Mosquito larvicidal

Aedes aegypti is referred to as yellow fever mosquito, which is a vector for communicating severe tropical fever. This mosquito is responsible for spreading dengue fever, chikungunya, zika virus and other disease. Thus, control of this mosquito larvae is required which is classically done by organophosphates and insect growth regulators (Kraemer et al., 2019). However, these

remedies have limitations including widespread damage to environment and emergence of resistance. Thus, to combat these problems, now emphasis is being posed over the use of phyto-herbal remedies. In this context, *P. longum* extract was examined, reporting 100% mortality against larvae. Further, pipernonaline was isolated and identified as the bio-active component of *P. longum* which was responsible for larvicidal action, although piperine, piperlongumine, and piperettine did not show any action against the larva (Yang et al., 2002). Nevertheless, plant products containing essential oils have been widely utilized to control mosquito species, thus further validation can be proceeded with the oil fraction of *P. longum*. Correspondingly, validation on other larvae like *Culex quinquefasciatus*, also needs to be evaluated.

3.3.23 Acaricidal

Rhipicephalus (Boophilus) microplus and *Hyalomma anatolicum* are the two ticks species which are responsible for spreading disease to cattle and humans. These ticks are distributed to hot and dry regions of India and are causing a lot of economic loss to dairy industry. The prevention of these ticks are done by so called acaricides and vaccination, but the growth of resistance are making demand for newer agents (Gaur et al., 2016). Aqueous and ethanol extract of *P. longum* is being examined for acaricidal activity against three-host ixodid tick, *Hyalomma anatolicum*. The maximum acaricidal property was displayed by the alcoholic extract of *P. longum* seeds with the minimum lethal concentration 50 (LC50) and lethal concentration 95 (LC95) values of 0.071% and 0.135% respectively (Singh et al., 2017). In another study, *P. longum* fruit extract was evaluated against adult engorged females of *Rhipicephalus (Boophilus) microplus* using adult immersion test. The extract displayed additional action on the reproductive physiology of ticks by inhibiting oviposition with 36.1–89.3% mortality (Godara et al., 2018). These findings suggest that this unconventional therapy for controlling cattle ticks would not only be useful for organic livestock production but could also be an alternative for controlling resistant strains.

3.3.24 Reproductive system

The evidence of *P. longum* to demonstrate contraceptive properties came from the fact that it's one of the Ayurvedic formulation referred as Pippaliyadi vati, and its individual constituents are used as a contraceptive for women since ancient times (Bhutani et al., 2007; Chaudhury et al., 2001). Thus, studies have been performed to validate the action of *P. longum* for infertility

action. It has been claimed that *P. longum* interferes with the regular progression of reproductive senescence and encourages infertility through gonadotropin inadequacy and variation of inflammatory intermediaries (Sarwar et al., 2014). Moreover, *P. longum* was also employed for spermicidal activity against human spermatozoa. The experiment stipulated that the hexane part of *P. longum* has an immobilizing factor that presumably lessens motility by rendering sperm non-viable via disturbing the layer engineering of the sperm cell (Sarwar et al., 2015). However, these findings are based on only two available studies and require validation by further experiments.

3.3.25 Anti-snake venom

Snake bites in tropical developing countries lead to serious clinical manifestations including hemorrhage, shock, acute kidney, and local tissue damage. Anti-venom therapy is mainly given through intravenous route, which is expensive and delay in their availability at the site of accident, leads to fatality. Thus, other measures are being considered which delays the onset of toxicity. Herbal medicines are gaining interest worldwide and help in revealing the symptoms of snakebite such as pain, anxiety, reducing local effects, produce anti-inflammatory action, thus preventing lethality (Parker-Cote and Meggs, 2018; Warrell, 2019). Fruit extract from *P. longum* was evaluated against snake venom in embryonated fertile chicken eggs, mice and rat. Inhibition of venom lethal action, hemorrhagic action, necrotizing action, defibrinogenating action, paw edema, mast cell degranulation, creatine kinase assay and catalase activity were examined and were brought to normal level at the doses of 250 mg/kg, 500 mg/kg and 750 mg/kg (Shenoy et al., 2013). This study provides evidence that plant extract can be used to reduce lethality and can be considered in rural areas where folk medications are accessible.

3.3.26 Bio-enhancer

The rate and extent of reaching a drug into systematic circulation is referred to as bioavailability. The drugs given by intravenous route demonstrate maximum bioavailability, though when drugs are given via oral route the extent to cross the cellular barrier decreases. Thus, decrease in bioavailability increase the dose required to produce action. Bioenhancers are the agents that increase the bioavailability and bio-efficiency of particular drug, in Ayurvedic terms bio-enhancer are referred to as Yogvahi (Alexander et al., 2014). In 1979, the term bioavailability

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enhancer was originally created by Indian scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine). They further isolated the world's first alkaloid as piperine from *P. longum* and *P. nigrum*. Also C. K. Atal, the director of Indian Institute of Integrative Medicine, screened a range of Ayurvedic herbs and found out the addition of trikatu or one its ingredient in wide range of ayurvedic formulation is due to their bioenhancer activity. Moreover, various mechanisms have also been put forward to analyze the bio-enhancer action of piperine, which comprises of DNA receptor binding, modulation of cell signal transduction and inhibition of drug efflux pump, inhibiting enzymes participating in biotransformation of drugs. It has been now ascertained that use of bio-enhancer will diminish the drug cost, toxicity, and other adverse effects, and provide favorable impact on the national economy (Atal and Bedi, 2010; Randhawa and Jagdev Singh Kullar, 2011). However, the major mechanism revealed by which *P. longum* show bioenhancer action is due to inhibition of P-gp efflux transporter present in gastrointestinal wall and principal metabolizing enzyme CYP3A4 (Harwansh et al., 2014). The utilization of bioenhancers will, in general, apply a positive effect on the economy of a nation by lessening the dose of the medication and thereby toxic effects.

3.4 Phytochemistry

Plants produces various metabolic products, the primary metabolic products are utilized as source of energy, while the secondary metabolites are either stored or employed for special functions like plant defense. These secondary metabolites cover a wide range of chemical constituents ranging from alkaloids, terpenoids, steroids, flavonoids to glycosides and fatty acid. It is because of these secondary metabolites which make the characteristic of the plant and are also responsible for medicinal purpose of extract (Foyer and Noctor, 2000). Piperine alkaloid is principle component in *P. longum* which accounts for 3-5% in the plant. The piperine content is responsible for pungent and hot flavor of the plant. *P. longum* also spectacles the occurrence of essential oil, bitter principle, arbutin, steroids, coumarins, alkaloids and sterols (sitosterol and stigmasterol) in *P. longum* extract (Saraf and Saraf, 2014). Table 3 summarizes the major phytochemicals that have been isolated and characterized from *P. longum*. Medicinal activities of *P. longum* are considered to be attributed mainly to its alkaloid content. Some of the chemical structures of secondary metabolites that have been isolated and characterized from *P. longum* are

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shown in Table No. 4, also Figure 5 represent important aspects of *Piper longum* and some of its pure bio-active constituents to understand the mechanism(s) of action.

Table No 3: Phytoconstituents of *Piper longum*

	Active constituent	Part of Plant	Reference
Alkaloid	Piperine, Piperlongumine and Piperlonguminine	Roots	Chatterjee and Dutta 1966; Chatterjee and Dutta 1967; Bao et al., 2012
	Dehydropipernonaline	Fruit	Shoji et al., 1986
	Cepharadione A, Cepharanone B, Aristolactam AII, norcepharadione A, 2-hydroxy-1-methoxy-4H-dibenzo[de, g]qumoline-4,5(6H)-dione, piperolactam A, piperolactam B 2-hydroxy-1-methoxy-6-methyl-4Hdibenzo[de, glqumoltne-4,5(6H)-dlone [piperadione]	Roots	Desai, et al., 1988
	(Z)-12-octadecenicalpha-glycerol monoester, Piperine, Beta-sitosterol, Daucosterol, Desmethoxypiplartine	Fruit	Zhang et al., 1996
	Tetrahydropiperine	Fruit	Madhusudhan and Vandana, 2000
	Coumaperine, Piperolactam A, Turmerone, Aphanamol, Bisdemethoxycurcumin, Demethoxycurcumin, 1-[1-oxo-5 (3,4-methylenedioxyphenyl) -2E,4E-pentadienyl] –pirrolidine, N-5-(4-hydroxy-3-methoxyphenyl)-2E-pentenoyl piperidine, 1-[1-oxo-5 (3,4-methylenedioxyphenyl) -2E-pentenyl] –pirrolidine, 1-[1-oxo-9 (3,4-methylene dioxyphenyl) -2E, 8Enonadienyl] –pyrrolidine, octahydro-4-hydroy- 3alpha-methyl-7 methylenealpha-(1-methylethyl)-1H-indene-1-methanol	Whole plant	Liu et al., 2009
	Retrofractamide C, Pipercide, Pellitorin, Dehydroretrofractamide C, Guineesine, Dehydropipernonaline, Piperloein B, P(2E,4Z,8E)-N-[9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl]-piperidine, pipernonaline	Fruit	Lee et al., 2010

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	Pipyahyine	Fruit	Madhu et al., 2011
	GB-N	Fruit	Bao et al., 2012
	Piperlongumamides A-C, Piperchabamide B,	Fruit	Yang et al., 2013
	Ethyl 3',4',5'- trimethoxycinnamate	Fruit	Kumar et al., 2005
	3β, 4α-dihydroxy-1-(3-phenylpropanoyl)-piperidine-2-one and (2E, 4E,14Z)-6-hydroxyl-N-isobutyleicosa-2,4,14-trienamide	Fruit	Jiang et al., 2013
Lignan	Sylvatin, Sesamin and Diaeudesmin	Seeds	Dutta et al., 1975
Essential oil	α -Pinene, Sabinene, Myrcene, δ-3-Carene, Limonene, a-Copaene, 6-Elemene, 0-Elemene, p-Caryophyllene, 9-Octadecene, p Selinene, a-Selinene, 6-Cadinene, Caryophyllene oxide	Fruit	Tewtrakul et al., 2000
	α-Pinene, β-Pinene, Limonene, Linalool, 1-Methylhexyl acetate Decanal, Bornyl acetate, 2-Undecanone, γ-Elemene, Copaene, β-Cubebene, β-Elemene, Dodecanal, Caryophyllene, Guaiene, Gurjunene, Humulene (Z)-â-Farnesene, γ-Muurolene, α-Muurolene, β-Patchoulene, Cadinene, Nerolidol, Elemol, Caryophyllene oxide, δ-Cadinol, α-Cadinol, β-Eudesmol, α-Eudesmol, 9-Eicosyne	Leaf	Varughese et al., 2016; Bhuiyan et al., 2008
	α-Pinene, Camphene, β-Pinene, β-Myrcene, β-Phellandrene, Limonene, cis-Ocimene, Terpinolene, Linalool, 1-Methylhexyl acetate, 2-Nonanone, Terpineol, 2-Undecanone, Caryophyllene, 2-Tridecanone, Bisabolene, Nerolidol	Fruit	Varughese et al., 2016
	β –Pinene, Camphene, β-Pinene, β-Myrcene, α-Phellandrene, β-Phellandrene, Limonene, Eucalyptol, trans-Ocimene, cis-Ocimene, Bornyl acetate, 2-Undecanone, Tridecane, δ- Elemene, Terpinyl acetate, β-Elemene, Caryophyllene, Humulene, 1-Pentadecene, Pentadecane, β-Patchoulene, Nerolidol, Caryophyllene oxide, δ-Cadinol, Heptadecane	Root	Varughese et al., 2016
	Bornyl acetate, 2-Undecanone, Tridecane, δ- Elemene, Terpinyl acetate, Caryophyllene, Gurjunene, Humulene, Pentadecane, β-Patchoulene, Nerolidol, Caryophyllene oxide	Stem	Varughese et al., 2016
Flavonoids	Catechin, Epicatechin, Quercetin, Myricetin, Kaempferol, Apigenin, Luteolin, Naringenin	Fruit	Mustafa et al., 2010
Amide	Sarmentine	Fruit	Huang et al., 2010

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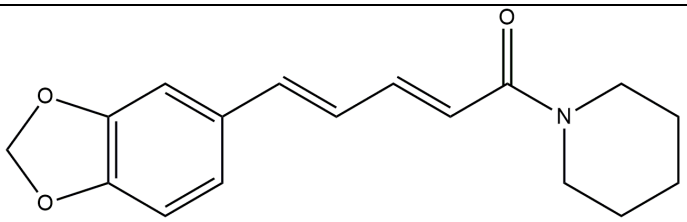
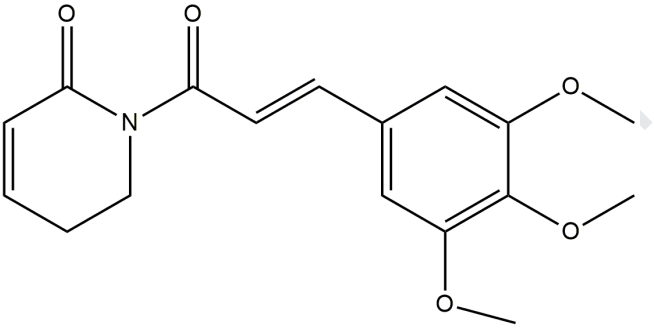
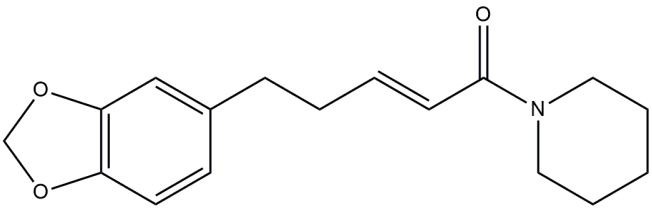
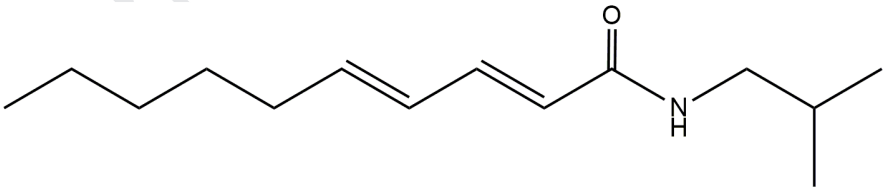
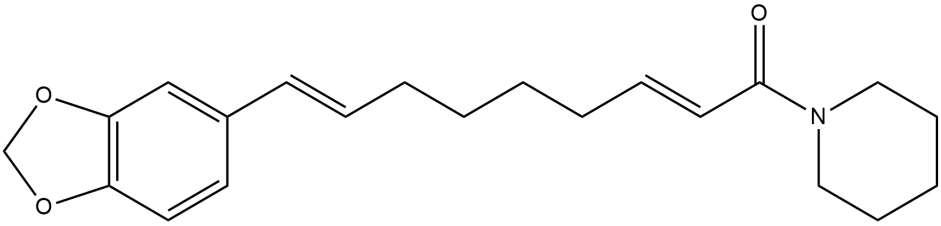
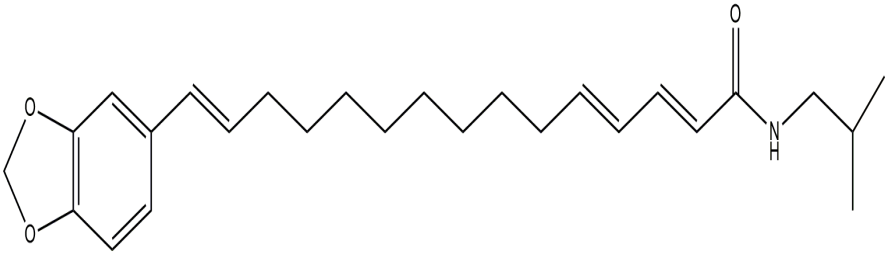
Table No 4: Chemical structures of the major constituents of *Piper longum*

S.No.	Isolated Moiety	Chemical Structure
1	Piperine	
2	Piperlongumine	
3	Piperanine	
4	Pellitorine	
5	Pipernonaline	
6	Guineensine	

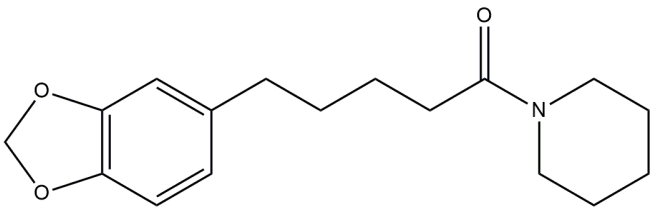
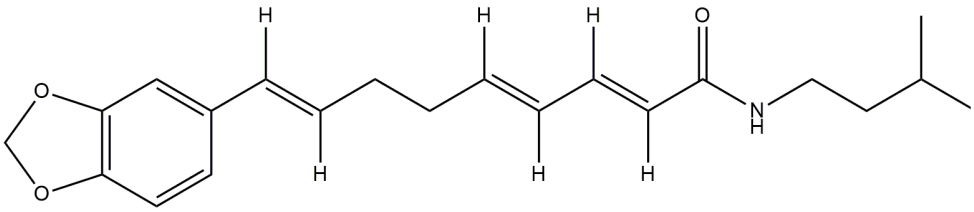
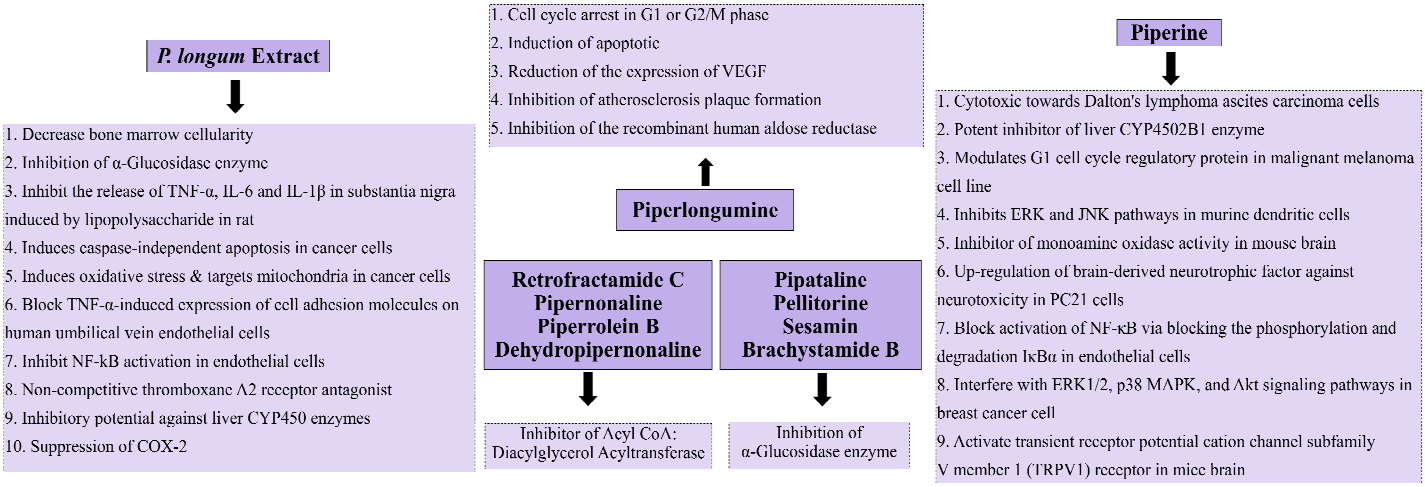
7	Tetrahydropiperine	
8	Pipyahyine	

Figure 5: Mechanism of action of *P. longum* extract and its isolated moieties.



References: Duan et al.,2016; Lee et al., 2010; Bancroft , 2002; Fofaria et al., 2014; Bae et al., 2010; Sunil and Kuttan, 2004; Chinta et al., 2015; Lee et al., 2006; Pullela et al., 2006; Iwashita et al., 2007; Bi et al., 2015; Ovadje et al.,2014; Vedhanayaki et al., 2003; Harwansh et al., 2014.

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3.4.1 Alkaloid

Alkaloids are the chief constitute in the Piper genus and piperine is the major secondary metabolite of *P. longum*. Piperine display characteristic mouthfeel, a pepper-like pungency and pronounced salivation and numbness (Peter, 2006). Below mentioned are the studies that have been performed on various parts of plant which resulted in the isolation of several amides from *P. longum*.

3.4.1.1 Whole Plant

A new amide, the dimer of desmethoxypiplartine, together with (Z)-12-octadecenicalpha-glycerol monoester, piperine, beta-sitosterol and daucosterol were isolated from *P. longum* (Zhang et al., 1996). Liu et al., has utilized the whole plant of *P. longum* for the isolation of compounds. They have prepared ethanol extract of *P. longum* and further extracted with petroleum ether, chloroform and butanol consecutively. Moreover, compounds were isolated by chromatographic technique from chloroform fraction and structure elucidation was done by MS, 1H-NMR, 13C-NMR. The compounds that were identified comprise of coumaperine, N-5-(4-hydroxy-3-methoxyphenyl)-2E-pentenoyl piperidine, piperolactam A, 1-[1-oxo-5 (3,4-methylenedioxyphenyl) -2E,4E-pentadienyl] -pirrolidine, 1-[1-oxo-5 (3,4-methylenedioxyphenyl) -2E-pentenyl] –pirrolidine, 1-[1-oxo-9 (3,4-methylene dioxyphenyl)-2E, 8Enonadienyl]-pyrrolidine, (R)-(-)-turmerone, octahydro-4-hydroy-3alpha-methyl-7-methylenealpha-(1-methylethyl)-1H-indene-1-methanol, (+) -aphanamol I, bisdemethoxycurcumin and demethoxycurcumin (Liu et al., 2009). Bao et al., have identified a piperine derivative called GB-N from ethanol extract of dried *P. longum*. They further evaluated its bio-efficiency and reported that GB-N could improve serum lipid profile in high-lipid diet-induced hyperlipidemic rats by regulating lecithin cholesterol acyltransferase, low-density lipoprotein receptor and CYP7A1 enzyme (Bao et al., 2012).

3.4.1.2 Fruits

Piperine and piperlongumine are the two major piperidone alkaloids which are found in *P. longum* (Chatterjee and Dutta, 1966, 1967). Piperine is the major alkaloid occurring in the Piper species. The yield of piperine was 16.58 ± 0.02 mg/g, obtained from the dried fruit of *P. longum*, which was collected from India, while piperine yield almost doubles from *P. nigrum*. Although

the yield of piperlonguminine was found to be 0.80 ± 0.02 mg/g which is highest in all Piper species (Bao et al., 2014). Dehydropipernonaline is one of the amide identified from the *P. longum* fruits, this amide display coronary vasodilating activity, as confirmed through *in vitro* bio-assay (Shoji et al., 1986). Madhusudhan and Vandana has reported the occurrence of tetrahydropiperine from the extract of dried fruit of *P. longum* which is natural arylpentanamide (Madhusudhan and Vandana, 2001). Kumar et al., have also isolated novel aromatic ester from *P. longum* fruit extract and identified the compound as ethyl 3',4',5'-trimethoxycinnamate, which seems to inhibit TNF-α-induced expression of ICAM-1 when tested using primary human umbilical vein endothelial cells, with maximum inhibition at the concentration of 50 µg/ml in a concentration dependent manner (Kumar et al., 2005). Park et al., have correspondingly isolated acidamides from the fruits of *P. longum* which includes piperine, pipernonaline, piperoctadecalidine, and piperlongumine. They have also investigated the action of isolated moieties for anti-platelet action induced by collagen, arachidonic acid, and platelet-activating factor. The most prominent action was produced by piperlongumine at the concentrations of 300, 150, 30 and 10 µM (Park et al., 2007). Another 10 alkamides were isolated from dried fruit of *P. longum*. The methanol extract followed by mass spectroscopic examination results in the identification of retrofractamide C, pipercide, pellitorin, dehydroretrofractamide C, guineesine, dehydropipernonaline, piperloein B, P(2E,4Z,8E)-N-[9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl]-piperidine, pipernonaline, piperine. These compounds were further tested for inhibition of IL-6 action. Moreover dehydropipernonaline, piperloein B and pipernonaline were found to produce most prominent inhibitory effect on Stat3-dependent luciferase activities induced by IL-6 (Lee et al., 2010). Sarmentine is another specific compound isolated from ethanolic fruit extract of *P. longum*, which was found to be phytotoxic and may serve as herbicide (Huang et al., 2010). Moreover, Madhu et al., has isolated pipyahyine from petroleum ether extract of fruit of *P. longum* which possess larvicidal activity against *Culex quinquefasciatus* (Madhu et al., 2011). Similarly, two new amides from dried fruit of *P. longum* were isolated. These moieties include piperlongimin A and piperlongimin B, along with other known compounds. The moieties were demonstrated to provide cytotoxic activity on HL-60 cell lines (Mishra et al., 2011). Piperlongumide, [N-isobutyl-19-(3′,4′-methylenedioxyphenyl)-2E,4E nonadecadienamide], 1-(3,4-methylenedioxyphenyl)-1E tetradecene, piperlongimin A [2E-Nisobutylhexadecenamide], 2E,4E-N-isobutyl-octadecenamide, piperlongimin B [2E-

octadecenoylpiperidine], 2E,4E-N-isobutyl-dodecenamide, and 2E,4E,12E,13-(3,4-methylenedioxyphenyl)-trideca-trienoic acid isobutylamide were the isolated moieties reported by Sahi et al., these compounds further seems to exhibit activity against *Leishmania donovani* (Sahi et al., 2012). Ghosal et al., have confirmed the presences of new alkaloid amide derivative called piperlongumide. This compound has been isolated from the hexane fraction of dried methanol extract of fruit of *P. longum*. Further, they have demonstrated the leishmanicidal activity of isolated compounds against promastigotes and axenic amastigotes of *Leishmania donovani* (Ghosal et al., 2012). Yang et al., have also isolated amide alkaloids which were identified as piperlongumamide A, piperlongumamide B and piperlongumamide C, as recognized for the first time. Although the other already known compounds which were isolated includes piperine, isopiperine, chavicine, piperlonguminine, pellitorine, brachystamide B, guineensine, piperchabamide B, (2E,4E)-N-dodecadienoylpiperidine, dehydropipernonaline pipernonaline and piperolein B. However, these isolated moieties were also found to possess cytotoxicity against human leukemia, human lung cancer, human breast cancer, human liver cancer and human rectal cancer (Yang et al., 2013). Jiang et al., has performed bioassay guided phytochemical investigation which resulted in isolation of 3β, 4α-dihydroxy-1-(3-phenylpropanoyl)-piperidine-2-one and (2E,4E,14Z)-6-hydroxyl-N-isobutyleicosa-2,4,14-trienamide. As identified by MS, IR, UV, 1D and 2D NMR data these moieties were isolated for the first time and further they were evaluated for anti-HBV *in vitro* assay. The isolated moiety showed notable activity in suppressing the secretion of hepatitis B virus e antigen, with an IC_{50} value of 0.21 mM in Hep G2 cell line (Jiang et al., 2013), however, reported IC_{50} value seems unrealistically high for this action. For better and efficient isolation Li et al., have developed two-dimensional liquid chromatography method coupled with normal phase liquid chromatography and reversed phase liquid chromatography. The isolated compounds were characterized by electrospray ionization-mass spectrometry and nuclear magnetic resonance spectroscopy. The isolated compounds include pellitorine, N-[(2E,4E)-Decadienoyl]-piperidine, N-Isobutyl-2E,4E-undecadienamide, piperlonguminine, dihydropiperlonguminine, piperanine, N-[(2E,4E)-Tetradecadienoyl]piperidine, N-Isobutyl-2E,4E-hexadecadienamide, retrofractamide A, pipercallosine, (2E,4E,12Z)-N-Isobutylocatadeca-2,4,12-trienamide, N-Isobutyl-2E,4E-octadecadienamide, 1-[9-(30,40-Methylenedioxyphenyl)-4E,6E,8Enonatrienoyl] piperidine, dehydropipernonaline, pipernonatine, (E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)non-2-

en-1-one, 1-(2E,4E,12E)-octadecatrinoylpiperidine, retrofractamide B, (2E,4E,14Z)-N-Isobutyleicosa-2,4,14-trienamide, N-Isobutyl-2E,4E-decyldecadienamide, (2E,4E,10E)-N-11-(3,4-Methylenedioxyphenylhmdecatrienoylpiperidine, piperchabamide B, 1-[(2E,4E,14Z)-1-Oxo-2,4,14-eicosatrienyl]-piperidine, guineensine, (2E,4E,14Z)-N-Isobutyldocosa-2,4,14-trienamide, (2E,4E,12E)-13-(Benzo[d][1,3]dioxol-6-yl)-1- (piperidin-1-yl)trideca-2,4,12-trien-1-one, (2E,4E,13E)-14-(Benzo[d][1,3]dioxol-6-yl)-Nisobutyltetradeca-2,4,13-trienamide and brachyamide B (Li et al., 2013). Nevertheless, Liu et al., have also developed a simple, operative and appropriate ultra-fast liquid chromatography combined with electrospray ionization tandem mass spectrometry (UFLC-ESI-MS/MS) method to isolate piperine, piperlonguminine, Δα,β-dihydropiperlonguminine, pellitorine and piperanine from *P. longum* (Liu et al., 2015).

The traditional methods employed to isolate amides are quite monotonous and require multiple chromatography steps. Thus, to obtain amides of high purity new technique need to be developed. Wu et al., has developed a new chromatographic technique using counter-current chromatography with upright type-J multilayer coil planet centrifuge for purification of fruits of *P. longum*. They have isolated the following compounds: (2E,4E)-N-Isobutyleicosa-2,4-dienamide, (2E,4E,14Z)-N-Isobutyleicosa-2,4,14-trienamide, (2E,4E,12Z)-N-Isobutylocatadeca-2,4,12-trienamide, guineensine, pipernonaline, pellitorine, piperine, piperanine and piperlonguminine (Wu et al., 2004). Abdubakiev et al., have isolated 16 N-Alkylamides from the fruits of *P. longum*, which consist of piperine, isopiperine, piperlonguminine, Δαβ-dihydropiperine, dihydropiperlonguminine, dehydropipernonaline, retrofractamide A, pipercide, guineensine, pipernonaline, retrofractamide C, trans-fagaramide, cis-fagaramide, piperodione, pellitorine, and (2E, and 4E,15Z)-N-isobutyl-eicosa-2,4,15-trienamide. Moreover piperine, isopiperine, piperlonguminine, retrofractamide A and retrofractamide C demonstrated strong ability to increase the melanin content and weak simulative effect on the tyrosinase activity (Abdubakiev et al., 2019).

3.4.1.3 Roots

The first evidence of identification of compounds from roots of *P. longum* initiated from the report of Chatterjee and Dutta, 1967. They have reported the presences of piperlongumine and piperlonguminine in the roots of *P. longum*, which was isolated from the petrol fraction of extract (Chatterjee and Dutta, 1967). Desai et al., have also isolated aristolactams and 4,5

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dioxoaporphines from the roots of *P. longum*. The ethanol extract of roots of *P. longum* yielded cepharadlone B, cepharadlone A, cepharanone B, aristolactam AH, norcepharadione B, and 2-hydroxy-1-methoxy-4H-dibenzo[de, g]qumoline-4,5(6H)-dione. Also three new alkaloids were characterized as 10-amino-4-hydroxy-3-methoxyphenanthrene-l-carboxyllc acid lactam [piperolactam A], 10-ammo-4-hydroxy-2,3-dimethoxyphenanthrene-1-carboxyhc acid lactam [piperolactam B] and 2-hydroxy-1-methoxy-6-methyl-4Hdibenzo[d,g]quinoline-4,5(6H)-dione [piperadione] (Desai et al., 1988).

3.4.2 Essential oil

Essential oil or volatile oil or ethereal oil are responsible for aroma of the plant and chemically belong to huge class of terpene family. Pharmacologically they are very important class of drug, as volatile oil is utilized in the treatment of stress, anxiety, depression, migrains, insomnia, as antibiotic and anti-microbial (Dhifi et al., 2016). *Piper longum* on distillation yields 0.7–1.5% of light green, viscous oil with a spicy odor resembling that of pepper and ginger(Peter, 2006).

3.4.2.1 Fruit

The dried fruits of *P. longum* on hydro-distillation yields monoterpene, sesquiterpenes and aliphatic hydrocarbon. Quantitatively *P. longum* fruit yielded 0.6% of oil content and 44 volatile oil components were identified. The major constituents of *P. longum* oil were found to be p-caryophyllene, aromatic-curcumene, germacrene D, 8 heptadecene, heptadecane, pentadecane and β bisabolene (Shankaracharya et al., 1997; Tewtrakul et al., 2000). Liu et al., has developed another method for extraction and isolation of volatile oil compounds. They have utilized microwave heating and solid phase microextraction followed by GC-MS for isolation of β-caryophyllene, β-pinene, 3-carene, D-limonene, eugenol, β-elemene, germacrene D, zingiberene, cadina-1,4-diene, β-eudesmol and cubenol were isolated (Liu et al., 2007).

As the composition of essential oil varies due to the geographic location, *P. longum* collected from Western Ghats of Kerala, India, were examined for the oil composition. GC/MS was employed to identify the components and it was found that essential oil consists of 0.1% of fresh weight of fruit. Also, 29 compounds were isolated which accounts for 97.1% of total oil. The principle components in fruit were β-pinene, camphene and α-pinene. As the composition of pinene and camphene were found in higher amount while eugenol and isoeugenol were not

observed in fruits. Moreover, the percentage of monoterpene were analyzed as 9.9 in root, 13.8 in stem and 12.4 in fruits, clearly indicating the highest amount in fruits. Another chief ingredient deducted in fruits were nerolidol and caryophyllene. The composition proportion of nerolidol diverse from 1.0 to 22.5, however that of caryophyllene from 5.6 to 16.8 in numerous parts. Caryophyllene is found to be distributed consistently through the plant. However, β-pinene was the single largest component in fruit accounting for 71.5% of oil (Varughese et al., 2016).

3.4.2.2 Inflorescences

Bhuiyan et al., has established the essential oil composition of *P. longum* inflorescences by GC-MS electron impact ionization method and reported 30 volatile compounds. The oil was rich in eugenol, caryophyllene, cinnamyl acetate, α-pinene, nerolidol, acetate, 2-heptanol, acetate, humulen-(v1), phytol, α-elemene, limonene and pinene (Bhuiyan et al., 2008).

3.4.2.3 Leaf

Bhuiyan et al., has also isolated 70 essential oil compound from the leaf of *P. longum*, which was rich in trans-nerolidol, caryophyllene, 3-heptene, 7-phenyl, benzyl benzoate, caryophyllene oxide, β-elemene, δ-guaiene, octacosane, nonane, 5-propyl, retinal, 2- heptanol, acetate, α-caryophyllene, cyclohexanone, 2-cyclohexylidene and lineoleoyl chloride (Bhuiyan et al., 2008). Varughese et al., have also determined the composition of volatile oil in leaf of *P. longum*. They have isolated 37 compounds from leaf which consist of 76.7% of total oil. Unlike to the fruits, leaf seems to contain sesquiterpene, apparently 68.7%, while the proportion of monoterpene was 2.6% (Varughese et al., 2016). Studies conducted by Utpala et al., has also led to the isolation of β- Caryophyllene, Nerolidol, alpha pinene, beta elemene, germacrene B, germacrene D from the leaf of *P. longum* (Utpala et al., 2014).

3.4.2.4 Roots

Varughese et al., has provided evidence that the essential oil composition of root and stem was minor than fruit and leaf of *P. longum*. Although the main components were comparable in root, stem and fruit. Upon isolation from roots, 38 compounds were identified which constitute 96.9% of total oil. The main components in roots of *P. longum* were identified as β-pinene, camphene

and α-pinene. However, the proportion of monoterpene was 62% while sesquiterpene hydrocarbons constitute 27.5 % of the total oil composition (Varughese et al., 2016).

3.4.2.5 Stem

Varughese et al., has also used stem for determination of essential oil composition of *P. longum*. They have found that volatile oil present in stem was similar to that of oil from root following the proportion of monoterpene as 69.2% while sesquiterpene with 25.6%. Also 36 compounds were identified which consist of 97.2% of total oil. The main compounds isolated in stem of *P. longum* were identified as β-pinene, camphene and α-pinene. Moreover the presences of higher proportions of monoterpene in root, stem and fruits were assumed due to the freshness of the sample (Varughese et al., 2016).

3.4.3 Lignans

Lignans are produced by oxidative dimerization of two phenylpropanoid units and consist of polyphenols. In plants, they are produced as a result of plants defense against stress and are served as anti-oxidant, anti-viral, anti-fungal and as anti-tumor for human utilization (Saleem et al., 2005). Although only a fraction of total chemical constituents of *P. longum* indicates the presence of lignans which are only existing in seeds.

3.4.3.1 Seeds

Dutta et al., has reported the presences of sylvatin, sesamin and diaeudesmin in the petroleum ether extract of seeds of *P. longum* (Dutta et al., 1975). Parmar et al., has also reported two lignin molecules as (+)-asarinine, pluviatilol (Parmar et al., 1998).

3.4.4 Thymoquinone

Thymoquinone is a monoterpene, present in oil of *Nigella sativa*. Chemically it consist of benzoquinone moiety and seems to modulate various molecular signals related to inflammatory and neurodegenerative process (Goyal et al., 2017). However, the presence of this moiety is not limited to *N. sativa*. When 50 species of Moroccan medicinal plant were evaluated for the identification of various phytochemicals, the presences of thymoquinone was identified in *P.*

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longum extract by performing electron spin resonance (ESR) spectroscopy (Mouhajir et al., 2001).

3.4.5 Phenolic and Flavonoids

Most of the plants with strong anti-oxidant potential is linked to phenolic compounds and flavonoids. These metabolites consist of aromatic ring with hydroxyl groups. They show action against many disorders including cancer, neurodegenerative disorder, aging, cardiovascular disorders etc, thus preventing and curing these aliments to promote human health (Tungmunnithum et al., 2018).

3.4.5.1 Fruit

Presence of flavonoid compounds was demonstrated by Bajpai et al., they have identified the caffeic acid, quercetin and kaempferol in the fruits of *P. longum* at the concentration of 829, 245 and 374 µg/g of plant material respectively (Bajpai et al., 2005). Similarly total phenolic content of *P. longum* was reported as 154.43 ± 0.14 mg gallic acid equivalent (GAE)/g, also the presences of flavonoids like catechin, epicatechin, myricetin, apigenin, luteolin and narngenin was confirmed (Mustafa et al., 2010). Ohno et al., have isolated prenylated phenolic compound called bakuchiol, bavachin and isobavachalcone from the fruits of *P. longum*. They have also established that the isolated moieties have inhibitory action against alpha melanocyte stimulating hormone induced melanin production (Ohno et al., 2010). The fruits of *P. longum* extract yielded apigenin 7, 4'-dimethyl ether from the ethyl acetate extract of this herb. The structure of this flavonoid was elucidated by spectroscopic means. Moreover this compound seems to confirm its action as anti-oxidant, anti-diabetic and anti-obesity action (Krishna et al., 2015).

3.4.5.2 Leaves

The leaves of *P. longum* also confirms the occurrence of total phenolic content by 18.19±0.4 mg/g gallic acid equivalent on dry weight basis (Bajpai et al., 2005).

3.4.6 Miscellaneous

Presences of dihydroxyphenolics and hydroxydopamine have been observed in the extract from *Piper longum* seeds (Mouhajir et al., 2001). D asparatic acid, cysteine, n-eicosane, p

methoxyacetophenone, n-Heneicosane, methyl 3,4,5 trimethoxycinnamate, n-octadecane, β phenylethanol, serine, n-triacontane, and L-tyrosine has also been reported in *P. longum* (Parmar et al., 1998).

3.5 Nanoformulation

The modern world is now relaying over nanoparticles for treatment of various disease, as they offer special advantage of organ targeting, enhanced bioavailability and many more. Although the use of non-bio-degradable substances makes the nanoparticles unfavorable for biological system as well as environment. Thus, to combat such problem, silver/gold nanoparticles are being formulated with natural substances to allow reduction of metal and make these formulations biodegradable. This type of technique where natural substances are employed for improvement of the nanostructure is referred to as green nanotechnology (Nath and Banerjee, 2013). Reddy et al., has attempted to prepare green synthesized silver nanoparticles by *Piper longum* fruit and evaluated its anti-bacterial, anti-oxidant and cytotoxic potential. Bio-green silver nanoparticles were synthesized and confirmed by UV spectrometer. The shape and size distribution of the synthesized particles were confirmed by SEM and DLS size analyzer, respectively. By the use of FT-IR, it was established that phenolic compounds present in the *P. longum* extract was responsible for stabilization of formulation. *P. longum* silver nanoparticles were assessed for anti-oxidant action by employing DPPH scavenging assay, superoxide scavenging activity, nitric acid quenching activity and H_2O_2 scavenging activity. The average percentage inhibition of oxidative action by these nanoparticles was observed as 67%, 60%, 70%, and 96% respectively for DPPH scavenging assay, superoxide scavenging activity, nitric acid quenching activity and H_2O_2 scavenging activity. The antibacterial action was examined on *B. cereus, S. aureus, Pseudomonas aeruginosa* and *Bacillus subtilis,* using streptomycin antibiotics as a reference drug. At the concentration of 20 µg/ml, it showed zone of inhibition against all the strains. *P. longum* silver nanoparticles were also evaluated for cytotoxicity using MTT assay and it was found that these nanoparticles reduced cell viability in MCF-7 cell line and the IC_{50} values were found to be 67 µg/ml and 51 µg/ml at 24 and 48 h respectively (Reddy et al., 2014). However, the chemical assays adopted for evaluating the anti-oxidant action in the above-mentioned study does not validate the biological effect, thus other methods will be required to establish such action. Also, the use of such assays restricts the development of

efficient drug delivery system as they do not provide enough therapeutic evidence for biological action. Further, the properties of bio-green nanoparticles may serve to a have broad range of action in biological system. In another study, gold nanoparticles were synthesized by green method using *P. longum* fruit extract. Moreover, this formulation has positive results in anti-oxidant assays and catalytic activity. These green synthesized nanoparticle may be useful in clearing toxic dyes from industrial wastage (Nakkala et al., 2016). A similar study was performed by Mallikarjuna et al., where they have used leaves extract of *P. longum* for reduction of gold ions in gold nanoparticles. Their study has demonstrated that on increasing the time of reaction, changes in morphology of gold structure was observed. They found that the spherical structure was changed to nanohexagons and triangles as confirmed by XRD and SAED patterns. Also, the nanoparticles were surrounded by layer of biomolecules which were responsible for the gold ion reduction (Mallikarjuna et al., 2015). Nasrollahzadeh et al., has also provided evidence in support of green synthesis of nanoparticles by using *P. longum*. They have prepared palladium nanoparticle via the reduction of aqueous Pd^{2+} ions consuming extract of fruits of *P. longum* deprived of any stabilizer or surfactant (Nasrollahzadeh et al., 2015). Nanoparticles designed by the chemical and physical methods have serious issue related to consumption of energy, time, non-economic and harmful to humans. Thus bio-green methods are used to synthesize them, one of which involves use of plant-based system. Bio-reduction is achieved by the use of plant extract to finally formulate the metallic nanoparticle. This method is straightforward, vigorous, ecological friendly, economic and has great potential (Shanker et al., 2016). The above evidence shows that *P. longum* extract can be efficiently used for green synthesis of nanoparticles.

4. Conclusion and Future directions

Various research groups have explored the medicinal utilization of the *P. longum* and some encouraging fundamental discoveries have been accounted for the same. Regardless of this, there has not yet been adequate follow-up to completely explain these revelations. Based on our systematic review, it can be inferred that requisite attention must be given to prospective beneficial effects of *P. longum* as anti-amebic, anthelminthic, anti-cancer and as anti-diabetic. This is because both traditional claims and scientific studies to validate those claims have been available in the literature. However, the proof-of-concept trials are still warranted to authenticate the same in clinical practice. In view of this, progress can be made to elucidate the

cellular/molecular mechanisms involved and further steps may be taken for clinical validation or towards phytopharmaceutical development. Though the promising pre-clinical data for effectiveness of *P. longum* is also available for PD, anti-fungal action, and as hepatoprotective, these actions have not been claimed in traditional literature and hence it is very important to re-confirm these actions through *in vitro* or pre-clinical experiments in order to proceed further.

We identify several gaps in our understanding of the applications of *P. longum* as listed below. The first gap is that despite the traditional use of *P. longum* in insomnia, dementia and epilepsy, as mentioned in literature, only few studies limited to *in vivo* models have been conducted to evaluate these effects. Therefore, exploring its potential in neurological disorders particularly epilepsy and dementia associated with Alzheimer's disease along with elucidating mechanisms underlying anti-epileptic and/or pro-cognitive effects of this plant would be necessary. *P. longum* has also been indicated traditionally to be utilized in diseases like rheumatoid arthritis and asthma, yet very little scientific evidence in their favor have been identified. Further, for diseases like spleen disorder, puerperal fever, leprosy and as cholagogue, no scientific data is available to validate their utilization. Thus, here we propose that, tremendous work is required related to these diseases to validate their traditional claims.

Secondly, the traditional use of *P. longum* is typically shared with other medicinal plants. Therefore, the probable interactions and synergistic action of *P. longum* with other ingredients of poly-herbal preparations should be investigated. In this perspective, even though the safety and well-tolerability of the plant have been reported by experimental studies, potential interactions with components of poly-herbal preparations of drugs remain unclear.

Thirdly, numerous studies addressed absorption, distribution and metabolism of active ingredients present in *P. longum*. However, data on the pharmacokinetic aspects of the whole extracts of the plant are scarce and the penetration capacity of plant's ingredients into the central nervous system is largely anonymous. Therefore, forthcoming experiments should be intensive on exploring mechanism of actions and pharmacokinetics of the extracts and active compounds of *P. longum* based on suitable animal models and realistic dosages.

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We suggest that future research should focus on exploring the activities of *P. longum* extract by molecular biology and omics (genomics, proteomics, metabolomics) approaches. Also, it is necessary to investigate the bio-active compound of *P. longum* through bioassay-guided isolation, chemical characterization, mechanisms of action and structure activity relationship by advanced chemical and pharmacological techniques. The evidence presented in this review on the expansion and exploitation of ethno-medical and scientific knowledge would hopefully lead to more efforts towards development of competent and safe *P. longum* based pharmaceuticals.

5. Acknowledgement

Authors are grateful to DST-SERB grant and Jamia Hamdard Sliver Jubilee Research Fellowship for financial support.

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Author contributions:

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VY conducted the literature survey and prepared the MS draft. AK provided critical and useful inputs and corrected the MS. DV helped in designing the review framework, structured, edited and finalized the review. All authors checked the final MS.

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