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A REVIEW ON THE POTENTIAL USE OF BLACK GINGER FROM EASTERN INDIA

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ABSTRACT: *Kaempferia parviflora* Wall. Ex Baker is commonly known as 'black ginger' which belongs to the family Zingiberaceae. These are mostly found in the subtropical and tropical areas of Asia. It bears various pharmacological properties such as anticancerous, anti-allergic, anti-inflammatory, antioxidant and antimicrobial. Upon the reports we reviewed the plant aromatic rhizomes contained important phytochemicals as terpenoids and flavonoids. Silica gel column chromatography and high performance liquid chromatography analysis has identified methoxyflavonoids in its rhizome extract which can be used in cosmetics as natural anti-aging agent. These extracts have shown anticancer activities against cervical cancer, pancreatic cancer, gastric ulcer, ovarian cancer *etc*. There are several reports on the cytotoxic activities of rhizome extracts against different cancer cells which are an alternative way for cancer therapy. Also these plants are depleting in forests for which major steps must be taken for its conservation thereby improving its drug yielding potential which would help in various medicinal ways. Thus, the present report deals with numerous pharmacological activities of this plant which has to be improved further to retain its quality for pharmaceutical uses.

INTRODUCTION: *Kaempferia parviflora* of Zingiberaceae family is an important ginger plant which is also known as Thai ginseng or krachai-dum. It is widely distributed in India, Bangladesh, Thailand, Myanmar, Cambodia, Burma [1]. In India it is distributed in the northeast regions of Assam, Manipur and Nagaland. It is also commonly called as 'black ginger' due to the colour of its rhizome having enormous medicinal values. *K. parviflora* is an herbaceous perennial plant having small fleshy black rhizomes with tuberous roots, erect leaves, and white small flowers with a purple outline at the margin.

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It can grow in moist soil and humid conditions temperature ranging from 30-35 degree celcius up to 90 cm in height [2]. Among the local people of Thailand, the herb is used for health promoting to treat colic disorders, peptic and duodenal ulcer [2]. Number of researches has been done in *K. parviflora* for its valuable medicinal properties in treatment of many diseases. So, this paper deals with the review of *K. parviflora* pharmacological properties, *in-vitro* culture, antimicrobial, anticancer, antioxidant, cytotoxicity, anti-inflammatory and phytochemical study.

Phytochemicals of *Kaempferia parviflora* Wall. Ex Baker: In some study, silica gel column chromatography and HPLC analysis were done to identify twelve methoxyflavonoids from extracts of ethanol and chloroform in *K. parviflora* rhizomes. It included tectochrysin, 5, 7- dimethoxyflavone , 7, 4′- dimethylapigenin, trimethylapigenin, 5-

hydroxy-3, 7-dimethoxyflavone, 3, 5, 7-trimethoxyflavone, 3, 7, 4′-trimethylkaempferol, tetramethylluteolin, 3, 5, 7, 4'- tetramethyl kaempferol, retusine, ayanin and penta-methylquercetin [3]. Quercetin 3, 5, 7, 3', 4'-pentamethyl ether (KPMF-8), found in *K. parviflora*, directly activates SIRT1 more effectively than resveratrol, enhancing its deacetylase activity for potential health benefits [4].

Pharmacological Properties of *Kaempferia parviflora* Wall. Ex Baker: There are few pharmacological studies done on the benefits and effectiveness of methoxyflavones, cellular metabolism, anticancer, vascular relaxation, cardioprotective, sexual enhancing, neuroprotective antiallergic, anti-inflammatory, antioxidative, anti osteoarthritis and antimicroorganism activity. These could be due to increase in its mitochondrial function and activated cGMP-NO signaling pathways [5].

Anticancer Activity: *K. parviflora* extracts possess anticancer compounds which is active against many cancer cells like cervical cancer, pancreatic cancer, gastric ulcer, ovarian ulcer. Studies of *in-vitro K. parviflora* Wall ethanolic extract has been done which showed apoptotic effects on HL-60 cells. These extracts suppressed HL-60 cell growth and viability. Apoptosis of HL-60 cell line involved activation of caspase-3 resulting in apoptosis [6]. Antiproliferative activity with superficial CO_2 fluid and ethanol against human gastric adenocarcinoma and cervical cancer cells was investigated. *K. parviflora* revealed potent antiproliferative activities in supercritical CO_2 fluid extracts (SFEs) than ethanol extracts against both the cell lines due to the presence of high concentration of polymethoxyflavones (PMFs) in SFEs than ethanol extracts respectively. Further it showed that 5, 7-dimethoxyflavone could be a potent anticancer PMF in *K. parviflora* [7]. *K. parviflora* extract also inhibited PANC-1 cancer cell colony formation [8]. *K. parviflora* suppresses EGF-induced IL-6 and disrupts IL-6/STAT3 signaling crucial for cervical cancer progression, making it a potential alternative anti-cancer agent [9]. In the study of Thaklaewphan *et al., K. parviflora* extract showed suppression of inflammatory cytokine and chemokine for tumor inhibition associated with macrophages. It showed that the *K. parviflora* rhizomes can be used against ovarian cell carcinoma [10]. This species of *Kaempferia* rhizome essential oil shows cytotoxic behaviour against C33A cell line [11]. The extract of *K. parviflora* was cytotoxic to ovarian cancer cell line when there was increase in its concentration. *K. parviflora* was active against cell proliferation, migration and invasion. *K. parviflora* might have the ability to inhibit MMP-2 (Matrix metalloproteinase 2) and MMP-9 (Matrix metalloproteinase 9) activity assayed by gelatin zymography. Also, cells treated with *K. parviflora* extract with nuclear labelling showed DNA fragmentation. The caspase-3, caspase-7, and caspase-9 induction shows *K. parviflora* to be responsible for cell death through intrinsic apoptotic pathway. Thus the antitumor activities of *K. parviflora* regulated through PI3K/AKT and MAPK pathways shows reduction in phosphorylation of AKT and ERK [12].

Similarly *K. parviflora* extract of oil, methanol, ethyl acetate and hexane were used for antiinternalization of *Helicobacter pylori* in HEp-2 cells and antibacterial activity. All others except oil exhibited antibacterial activity with minimum inhibitory concentration. Above all ethyl acetate extract was most active that exhibited anti-internalization activity by inhibiting both virulent and non-virulent *H. pylori* strains in HEp-2 cells. Thus, it can be used as a potential herb for prevention in *H. pylori* [13].

Anti-inflammatory Activity: The gas chromatography-mass spectrometry showed few compounds present in *K. parviflora* like 5, 7-dimethoxyflavone, 5-hydroxy-3, 7, 3′, 4′-tetramethoxyflavone, 3, 5, 7-tri-methoxyflavone, 5-hydroxy-7, 4′-dimethoxyflavone, 3, 5, 7, 4′-tetramethoxyflavone having antiinflammatory activity when tested in basophilic leukemia cells of rats. It was found that DMF and TMF were more potent inhibiting antigen induced degranulation [14]. Green synthesis of gold nanoparticles using K. parviflora plant extracts is eco-friendly and cost-effective, offering anti-inflammatory properties[15].

Anti-allergy Activity: *K. parviflora* extract obtained polymethoxyflavones which inhibited RBL-2H3 cell degranulation. Those polymethoxy-flavones suppressed the antigen which induced cell degranulation. Further 5-Hydroxy-3, 7, 4′, -

trimethoxyflavone and 5, 3′- dihydroxy-3, 7, 4′,- trimethoxyflavone showed inhibiton by suppression of calcium as 2, 5-ditert-butylhydroquinone, promoting calcium from outside cells and endoplasmic reticulum [16]. There are reports on bioassay fractionation which led to methoxyflavone isolation from *K. parviflora* extracts using spectroscopic methods. Above all 5-hydroxy-3, 7, 3', 4'-tetramethoxyflavone bears good antiallergic activity against antigen induced hexosaminidase release as a marker of degranulation in RBL-2H3 cells with an decrease IC50 value followed by 5-hydroxy-7-methoxyflavone and 5-hydroxy-7,4 -dimethoxyflavone, where others showed moderate activities. Other compounds were determined for ionomycin induced hexosaminidase release mechanism. Thus results the mechanism of some compounds on inhibition of cell degranulation mainly involved inhibition of Ca^{2+} influx to the cells [17].

Antioxidant Activity: *K. parviflora* is a herbal medicine having beneficial properties for humans. They studied the anti-inflammatory properties of *K. parviflora* extracts. They studied the U.V light response when induced in mouse skin tissue for COX-2(Cyclooxygenase) expression. Thereby the activity was seen in *in-vitro* models caused by the antioxidative effect MAPK pathways activated by UV rays and repressed in *K. parviflora* treatment. Further, the role of oxidative stress in UV induced COX2 expression. Out of all polyphenols identified, gallic acid followed by apigenin and tangeretin were identified as major polyphenols respectively [18].

The lipophilic metabolites as fatty acids, carotenoids, phytosterols, tocopherols and vitamin K1 and are useful antioxidants in food, nutraceutical industries and cosmetics. Similarly the leaves of *Kaempferia* have been reported for containing several bioactive metabolites. Their study revealed the lipophilic antioxidant profile of *K. parviflora* leaves grown *in-vitro* and *ex-vitro* condition. Those compounds were tocopherols, phytosterols vitamins and carotenoids quantified by LC–MRM–MS(liquid chromatography-multiple reaction monitoring-mass spectrometry) method. The fatty acid in leaves was identified by GC–FID/MS (Gas chromatography-flame-ionization detection/mass spectrometry) methods. The leaves of *ex vitro* had high levels of α-carotene, β-carotene, α-tocopherol, α-linolenic acid, palmitic acid, palmitoleic acid, oleic acid, neoxanthin, lutein, violaxanthin as compared to the *in-vitro* leaves. Their result indicated the *ex-vitro* leaves can be used as natural valuable source for extracting lipophilic antioxidants [19].

Cytotoxic activity: Mala *et al.,* studied *K. parviflora* roots ethanolic extract on *S. mutans* KPSK2 biofilm formation by crystal violet assay. The cytotoxicity on human gingival fibroblast was studied by MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. It showed inhibition in biofilm formation at different concentrations. Hence it is potential and useful anti-biofilm agent against bacteria [20].

Antimicrobial activity: *K. parviflora* ethanolic extract showed antimicrobial activity against bacteria, yeast, dermatophyte fungi and human pathogens by agar disc diffusion methods. It showed strong antifungal activity against dermatophytes at a concentration of 2 mg/disc but no activities against bacteria and yeasts. It showed strong antifungal activity against *Trichophyton mentagrophytes, Microsporum gypseum* and *Trichophyton rubrum,* with different MIC(Minimum inhibitory concentration) values respectively. So *K. parviflora* ethanol extract could be traditionally used for the treatment of dermatophyte infections [21].

Tissue Culture of *Kaempferia parviflora* Wall. Ex Baker: The *K. parviflora in vitro* plantlets produced were used for microrhizome development. About 60% plants produced microrhizomes in the liquid media supplemented with BA (1mg/l) + NAA (1mg/l) and sucrose (60g/l) producing highest as 265mg/plantlet. This protocol would be used in *K. parviflora* microrhizome production for commercialization [22]. In the study of Prathanturarug *et al., K. parviflora* micropropagation protocol has been established using plant growth regulators. The rhizome buds were cultured on Murashige and Skoog basal media with 6-benzylaminopurine for eight weeks and then transferred to only MS media for four weeks. Then they obtained highest of 47.3 ± 3.4 shoots per explant followed by rooting and field transfer [23]. Plant regeneration *in-vitro* from *Kaempferia*

rhizome buds has been done for obtaining secondary metabolites with antioxidants. The silver oxide nanoparticles were used as disinfectant on rhizomes and plant hormones effect on shoot and root multiplication was noted. The surface sterilization using sodium hypochlorite was insufficient for which silver oxide nanoparticle was used without influence on its survival. The maximum number of shoot was found in 6-Benzyladenine and thidiazuron compared with others. The maximum number of roots and root length was observed in indole-3-butyric acid. After acclimitization 98% survivality was seen when plantlets were transferred to greenhouse. Liquid chromatography with tandem mass spectrometry were used for chemical profiling in leaf extracts and showed the presence of flavonoids in both *ex-vitro* and *in-vitro* rhizomes. They found 40 in *in-vitro* and 36 compounds in *ex-vitro* grown leaf samples, respectively. Also high acetyl-cholinesterase inhibitory activity was seen in greenhouse leaves whereas high butyryl-cholinesterase inhibitory activity was found in *in-vitro* culture leaves. Hence the leaves could be an alternative for bioactive compounds extraction [24]. Reports are available in successful establishment of *K. parviflora* suspension culture. The combination of 2, 4 dichlorophenoxyacetic acid (0.2 mg/L) and napthaleneacetic acid (0.2 mg/L) showed highest induction of callus. After callus transfer to 2, 4-D (1 mg/L) the proliferation rate was maximum. Also the embryogenic callus was found highest when suspended in liquid medium containing 2, 4 D (1 mg/L) [25].

Other Medicinal Properties of *Kaempferia parviflora* Wall. Ex Baker: *K. parviflora* cholinesterase inhibitory screening exhibited significant acetylcholinesterase and butyryl-cholinesterase inhibitory activities. There were atleast 13 known methoxyflavones isolated and structure analysis was done by NMR method. For the first time new compounds were present in this plant species. The highest inhibitors towards acetylcholinesterase and butyrylcholinesterase were 5, 7, 4¢-trimethoxyflavone and 5, 7-dimethoxyflavone with range 43–85% inhibitory activity. The structure-activity relationship showed compounds bearing 5, 7-dimethoxy groups and a free substituent at C-3 had significant inhibitory effect but those bearing a 5-hydroxyl group reduced the inhibitory potency respectively. On the other side flavones bearing 3¢- or 5¢-methoxy group did n't influence the inhibition. Hence this report would be helpful in treatment of Alzheimer's disease [26]. The toxicity and genotoxicity studies suggest that rhizome extracts of *K. parviflora* is safe or dietary use, as a functional food or supplement [27].

Other studies showed the UV-blocking and antioxidant properties of ethanolic extracts from *K. parviflora* [28]. Some findings indicate that ethanol extracts of *K. parviflora* have the potential to serve as novel drugs for Plasmodium and Toxoplasma infections [29].

The maceration of *Kaempferia* rhizome with methanol, ethanol, hexane and chloroform were used for adaptogenic activities using swimming test in mice. The hexane extracts showed shorter mouse immobilisation than control and was separated using column chromatography method. Among all fractions, terpenoids rich resulted highest adaptogenic activity as compared to root powder used as control. Terpenes present in the fraction could be attributed in decreasing the mice swimming exhaustion. Also no effects in weight, liver, heart, adrenal glands and kidneys of mice. The major constituents were found by GC–MS and NMR analysis as a copaene, b-elemene, E-caryophyllene and germacene D respectively [30].

TABLE 1: RESEARCH WORKS ON *KAEMPFERIA PARVIFLORA*

Medicinal activities of *Kaempferia parviflora*	Year	References
Bioactive flavonoids from *Kaempferia parviflora*	2004	(2)
Kaempferia parviflora Extract Inhibits STAT3 Activation and Interleukin-6 Production in HeLa Cervical Cancer Cells	2019	(9)
Mass propagation of *Kaempferia parviflora* Wall.ex Baker by *in vitro* regeneration	2007	(23)
Kaempferia parviflora and Its Methoxyflavones: Chemistry and Biological Activities	2018	(5)
Ethanolic rhizome extract from *Kaempferia parviflora* Wall. ex. Baker induces apoptosis in HL-60 Cells	2008	(6)
Green synthesis of gold nanoparticles using *Kaempferia parviflora* rhizome extract and their characterization and application as an antimicrobial, antioxidant and catalytic degradation agent	2021	(15)

Anti-allergic activity of compounds from *Kaempferia parviflora*	2008	(17)
Antimicrobial activity of the ethanol extract and compounds from the rhizomes of *Kaempferia parviflora*	2008	(21)
Anticholinesterase activity of 7-Methoxyflavones isolated from *Kaempferia parviflora*	2009	(26)
The effects of *Kaempferia parviflora* on anti-internalization activity of *Helicobacter pylori* to Hep-2 cells	2010	(13)
Quercetin 3, 5, 7, 3′, 4′-pentamethyl ether from *Kaempferia parviflora* directly and effectively activates human SIRT1	2021	(4)
Kaempferia sp. Extracts as UV protecting and antioxidant agents in sunscreen	2020	(28)
Adaptogenic active components from *Kaempferia parviflora* rhizomes	2012	(30)
Antiproliferative activity and polymethoxyflavone composition analysis of *Kaempferia parviflora* extracts	2012	(7)
Toxicological evaluation of standardized *Kaempferia parviflora* extract: Sub-chronicand mutagenicity studies	2019	(27)
Identification and evaluation of anti-inflammatory compounds from *Kaempferia parviflora*	2014	(14)
Establishment and optimization growth of shoot buds derived callus and suspension cell cultures of *Kaempferia parviflora*	2014	(25)
Ethanol Extracts from Thai Plants have Anti-Plasmodium and Anti-Toxoplasma Activities In Vitro	2019	(29)
Anti-allergenic activity of polymethoxyflavones from *Kaempferia parviflora*	2015	(16)
In vitro microrhizome formation in *Kaempferia parviflora*	2015	(22)
Recent Advances in *Kaempferia* Phytochemistry and Biological Activity: A Comprehensive Review	2019	(11)
Anti-cancer effects of *Kaempferia parviflora* on ovarian cancer SKOV3 cells	2018	(12)
Effect of *Kaempferia parviflora* on *Streptococcus mutans* biofilm formation and its cytotoxicity	2018	(20)
Suppressive effects of methoxyflavonoids isolated from *Kaempferia parviflora* on inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells	2011	(3)
Antiskin inflammatory activity of black ginger(*Kaempferia parviflora)* through antioxidative activity	2018	(18)
Anti-austerity activity of Thai medicinal plants: Chemical constituents and anti-pancreatic cancer activities of *Kaempferia parviflora*	2021	(8)
Analysis of lipophilic antioxidants in the leaves of *Kaempferia parviflora* Wall. Ex Baker using LC-MRM-MS and GC-FID/MS	2021	(19)
Kaempferia parviflora extract inhibits TNF-α-induced release of MCP-1 in ovarian cancer cells through the suppression of NF-κB signaling	2021	(10)
A review on the ethnomedicinal uses, phytochemistry, and pharmacology of plant species belonging to *Kaempferia* L. genus (Zingiberaceae),	2021	(1)
Establishment of a rapid micropropagation system for *Kaempferia parviflora* Wall. Ex Baker: Phytochemical analysis of leaf extracts and evaluation of biological activities	2021	(24)

RHIZOMES ***KAEMPFERIA PARVIFLORA* PLANT**

LEAVES **FLOWER**

FIG. 1: REPRESENTING *KAEMPFERIA PARVIFLORA* WALL. EX BAKER PLANT, RHIZOMES, LEAVES AND FLOWER

CONCLUSION: There are various medicinal plants having traditional uses even in the developing countries. Due to the depletion of these plant species in forests, necessary steps for documentation of rhizome quality and germplasm conservation must be taken. Based on the present

review, leaves, rhizomes and its roots have been widely used for its medicinal properties due to richness in terpenoids, flavonoids and other phytochemicals. As the plant is beneficial in various ways, we should conserve it by tissue culture method for future development.

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