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Qualitative and Quantitative Study of Phyto-Constituents and Antioxidant Potential of Rhizomes of *Kaempferia galanga*, *Kaempferia parviflora* and *Kaempferia pulchra*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Medicinal plants are the potent source of biologically active compounds and have always been of field of interest for the effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. The objective of this study was to screen the phytochemicals, estimate the content of phenolic, flavonoids and alkaloids compounds and determines the antioxidant capacity of the rhizomes *of Kaempferia galanga* Linn*, Kaempferia parviflora* and *Kaempferia pulchra* (*K. galanga, K. parviflora* and *K, pulchra,* Zingiberaceae). Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol, flavonoids and alkaloids were determined by the well-known test protocol available in the literature. The ethanolic extract of rhizomes of *K. galanga, K. parviflora* and *K, pulchra* was studied for antioxidant activity on different *in vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay and Nitric oxide (NO) radical scavenging method. Phytochemical analysis of ethanolic extract of all three plants revealed the presence of flavonoids, alkaloids, saponins, phenolics, carbohydrate, and tannin. The total phenolic, flavonoids and alkaloids content of ethanolic extract of *K. galanga, K. parviflora* and *K, pulchra* rhizomes were 0.813, 1.146; 1.047, 1.237, 0.285; 0.755, 0.822, 0.975/100mg respectively. All extracts showed dose dependent free radical scavenging property in the tested models, which was comparable to that of ascorbic acid. The present study concluded

that the crude extract of *K. galanga, K. parviflora* and *K, pulchra* rhizomes is a rich source of secondary phytoconstituents which impart significant antioxidant potential. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants will be very useful in the curing of various diseases when taken along with our food.

Keywords: Kaempferia galangal; Kaempferia parviflora; Kaempferia pulchra; Zingiberaceae.

1. INTRODUCTION

Herbal medicines have become more popular in the treatment of any diseases due to the popular belief that green medicine is safe, easily available and with fewer side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is a lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use [1]. The medicinal properties of some plants have been documented by some researchers [2-4]. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products [5]. Extraction and characterization of several phyto compounds of these green factories have given birth to some high activity profile drugs [6]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today face either extinction or less of genetic diversity [7]. Knowledge of the chemical constituents of the plant is desirable because such information will be valuable for the synthesis of complex chemical substances. Reactive oxygen species (ROS) or oxygen free radicals can cause damage to cells and tissues during infections and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases, like Alzheimer's disease, mutations and cancer [8,9]. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) have been restricted because of serious concerns about their carcinogenic potential [9,10]. Natural antioxidants, especially phenolics and flavonoids, are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [11]. Numerous studies were carried out on plants with antioxidant properties [10-12]. However, there is still great interest in finding new antioxidants from natural sources. *K. galanga* is an important medicinal plant and has been traditionally used in tropics and subtropics of Asia including Bangladesh, India, China, Japan and Indo-China [13]. *Kaempferia* is a genus of about 70 species of rhizomatous, aromatic perennial herbs found in Africa and South East Asia [14]. In India, it is cultivated mainly in the states of Tamil Nadu, Kerala, Karnataka and West Bengal [15]. The rhizomes of this plant are widely used in East Asia for a wide range of traditional and modern medicinal applications. It promotes digestion and cures rheumatism, asthma, headaches, cough, toothaches, skin disease, piles, fever, epilepsy, splenic disorders and wounds. The rhizomes contain alkaloid, starch, gum, fatty matter with a fragrant essential oil [16]. Pharmacological properties such as anti-inflammatory and analgesic, antidiarrhoeal, nematicidal, mosquito repellent and larvicidal, vasorelaxant, sedative, antineoplastic, antimicrobial, anti-oxidant and cytotoxic activity has been reported [17]. *K. parviflora* or Krachaidam is originally found in the North and Northeast of Thailand. The rhizomes of *K. parviflora*, also known as black ginger are popular as health-promoting herbs and traditionally used as a folk medicine for managing a variety of diseases, including inflammation, ulcers, gout, colic disorder, abscesses, allergy, and osteoarthritis [18,19]. A number of pharmacological researches on *K. parviflora* have claimed the valuable benefits for a variety of diseases. *K. parviflora* has recently been reported to possess antimycobacterial, antiplasmodial, anti-peptic ulcer and anti-viral protease effects as well as modulators of multidrug resistance in cancer cells [20]. *K. pulchra* a perennial herb is cultivated in some tropical countries including Myanmar, Indonesia, Malaysia and Thailand. It is commonly known as Shan-pan-oot and has been extensively used for cough, blood stimulation, quenching heat, urinary tract infection and mellitus diabetes and as a carminative, deodorant, and diuretic. It has been reported to possess antiinflammatory and antitumor activities. The rhizomes have been used locally for self-medication by cancer and AIDS patients. Previous studies reported the presence of sandar-acopimaradiene diterpenoids and ethyl 4-methoxy-trans-cinnamate [21-24]. Despite the immense ethno-medicinal properties attributed to *K. galanga, K. parviflora* and *K, pulchra*, the reported phyto-pharmacological

study on variety levels of these plants is relatively infrequent to the best of our knowledge. Therefore, the present study was aimed to evaluate and compare antioxidant activity of the ethanolic extracts of *K. galanga, K. parviflora* and *K, pulchra*, by using classical in-vitro assays for the purpose of validating its ethno medicinal use.

2. MATERIALS AND METHODS

2.1 Plant Material

Rhizomes of *K. galanga, K. parviflora* and *K, pulchra* were collected from rural area of Bhopal in the month of Dec. 2019. Plant material (rhizomes part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2 Chemical Reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

2.3 Defatting of Plant Material

The shade dried plant material was subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

2.4 Successive Extraction with Different Solvents

45.6 gm dried powdered of *K. galanga,* 52.4 gm dried powdered of *K. parviflora* and 40 gm dried powdered of *K. pulchra* have been extracted with chloroform, ethyl acetate, ethanol and aqueous using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis [25].

2.5 Phytochemical Screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [26,27]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

2.6 Total phenol Determination

The total phenolic content was determined using the method of Olufunmiso *et al* [28]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

2.7 Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [28]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

2.8 Total Alkaloids Determination

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered [29]. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent

blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100 mg of extract.

2.9 Antioxidant Activity

2.9.1 DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Olufunmiso *et al.,* 2011 [28]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

2.9.2 Nitric oxide (NO°) radical scavenging assay

The determination of NO° radical scavenging ability of the extracts is based on the inhibition of NO^{o} radical generated from sodium nitroprusside in phosphate buffer saline solution by Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Scavengers of nitric oxide act against oxygen, prompting to lessened production of nitrite ions which can be monitored at 546 nm [30]. Briefly, sodium nitroprusside (0.6 ml, 5 mM) solution was mixed with and without varying the concentration of the extracts or Ascorbic acid (2 ml, 10-200µg/ml) and incubated at 25 ± 2°C for 5 h. Incubated solution (2 ml) was mixed with equal volume of Griess reagent and absorbance of the purple colored azo dye chromophore was measured at λmax 546 nm using UV-Vis spectrophotometer. The NO^{o} radical scavenging ability was calculated using following formula:

$$\text{Scavenging activity } (\%) = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

3. RESULTS AND DISCUSSION

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the rhizomes of *K. galanga, K. parviflora* and *K, pulchra* using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder rhizomes of *K. galanga, K. parviflora* and *K, pulchra* are shown in Table 2-4. Ethanolic extract of all three plants revealed the presence of flavonoids, alkaloids, saponins, phenolics, carbohydrate, and tannin. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.011X+0.011, R^2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.032X + 0.018, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: Y=0.007X+ 0.024, R^2=0.995, where X is the Atropine equivalent (AE) and Y is the absorbance. The total phenolic, flavonoids and alkaloids content of ethanolic extract of *K. galanga, K. parviflora* and *K, pulchra* rhizomes were 0.813, 1.146; 1.047, 1.237, 0.285; 0.755, 0.822, 0.975/100mg respectively Tables 5-7. DPPH radical scavenging assay measured hydrogen donating nature of extracts [31]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *K. galanga, K. parviflora* and *K, pulchra* ethanolic rhizomes extract was found to be 58.79, 34.69 and 83.01µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed Table 8. Extracts showed NO^{o} scavenging effects by competing with oxygen to react with NO directly

hence inhibited the nitrite ion formation [32]. *K. galanga, K. parviflora* and *K, pulchra* ethanolic rhizomes extract showed nitric oxide (NO^{o}) radical scavenging activity with IC_{50} value of 90.36, 65.65 and 106.50µg/ml as compared to that of ascorbic acid (IC_{50} 24.63µg/ml) Table 9.

Table 1. Results of percentage yield of *K. galangal, K. parviflora* and *K. pulchra*

S. No.	Extracts	(%) yield *K. galangal*	(%) yield *K. parviflora*	(%) yield *K. pulchra*
1.	Chloroform	2.36	2.88	3.65
2.	Ethyl acetate	5.42	5.74	4.47
3.	Ethanol	7.69	9.68	7.16
4.	Aqueous	8.44	11.27	6.53

Table 2. Result of phytochemical screening of extracts of *K. galangal*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids				
	A) Hager's Test:	-Ve	-Ve	-Ve	-Ve
2.	Glycosides				
	A) Legal's Test:	-Ve	-Ve	-Ve	-Ve
3.	Flavonoids				
	A) Lead acetate Test:	-Ve	-Ve	+Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	-Ve	+Ve	-Ve
4.	Saponins				
	A) Froth Test:	-Ve	+Ve	+Ve	+Ve
5.	Phenolics				
	A) Ferric Chloride Test:	-Ve	-Ve	+Ve	+Ve
6.	Proteins				
	A) Xanthoproteic Test:	-Ve	-Ve	-Ve	-Ve
7.	Carbohydrate				
	A) Fehling's Test:	+Ve	-Ve	+Ve	+Ve
8.	Tannin				
	A) Gelatin test:	+Ve	+Ve	+Ve	+Ve

Table 3. Result of phytochemical screening of extracts of *K. parviflora*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids				
	A) Hager's Test:	-Ve	-Ve	+Ve	-Ve
2.	Glycosides				
	A) Legal's Test:	-Ve	-Ve	+Ve	+Ve
3.	Flavonoids				
	A) Lead acetate Test:	-Ve	+Ve	-Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	-Ve	+Ve	-Ve
4.	Saponins				
	A) Froth Test:	-Ve	-Ve	+Ve	+Ve
5.	Phenolics				
	A) Ferric Chloride Test:	-Ve	+Ve	+Ve	+Ve
6.	Proteins				
	A) Xanthoproteic Test:	-Ve	-Ve	-Ve	-Ve
7.	Carbohydrate				
	A) Fehling's Test:	+Ve	-Ve	+Ve	-Ve
8.	Tannin				
	B) Gelatin test:	-Ve	-Ve	+Ve	+Ve

Table 4. Result of phytochemical screening of extracts of *K. pulchra*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids				
	A) Hager's Test:	-Ve	-Ve	+Ve	+Ve
2.	Glycosides				
	A) Legal's Test:	-Ve	-Ve	-Ve	-Ve
3.	Flavonoids				
	A) Lead acetate Test:	+Ve	+Ve	+Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	-Ve	+Ve	+Ve
4.	Saponins				
	A) Froth Test:	-Ve	-Ve	+Ve	+Ve
5.	Phenolics				
	A) Ferric Chloride Test:	-Ve	-Ve	+Ve	-Ve
6.	Proteins				
	A) Xanthoproteic Test:	-Ve	-Ve	-Ve	-Ve
7.	Carbohydrate				
	A) Fehling's Test:	-Ve	-Ve	-Ve	-Ve
8.	Tannin				
	A) Gelatin test:	-+Ve	-Ve	+Ve	-Ve

Table 5. Estimation of total phenolic, flavonoids and alkaloid content of *K. galangal*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	-	-
2	Ethyl acetate	-	-	-
3	Ethanol	0.813	1.146	-
4	Aqueous	0.572	0.490	-

Table 6. Estimation of total phenolic, flavonoids and alkaloid content of *K. parviflora*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	-	-
2	Ethyl acetate	0.559	0.981	-
3	Ethanol	1.047	1.237	0.285
4	Aqueous	0.823	0.563	-

Table 7. Estimation of total phenolic, flavonoids and alkaloid content of *K. pulchra*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	0.134	-
2	Ethyl acetate	-	0.465	-
3	Ethanol	0.755	0.822	0.975
4	Aqueous	-	0.357	0.681

Table 8. % Inhibition of ascorbic acid and ethanolic extract of *K. galangal*, *K. parviflora* and *K. pulchra* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition			
		Ascorbic acid	*K. galangal*	*K. parviflora*	*K. pulchra*
1	10	44.65	30.11	35.41	28.66
2	20	48.62	39.57	45.89	30.87
3	40	65.34	42.98	57.22	39.24
4	60	69.65	49.77	60.55	43.51
5	80	77.41	57.32	68.77	46.55
6	100	84.13	65.24	69.32	56.44
IC 50		17.68	58.79	34.69	83.01

Table 9. Inhibition of ascorbic acid and ethanolic extract of *K. galangal*, *K. parviflora* and *K. pulchra* using NO method

S. No.	Concentration (µg/ml)	% Inhibition			
		Ascorbic acid	*K. galangal*	*K. parviflora*	*K. pulchra*
1	20	47.70	20.47	31.22	15.64
2	40	52.92	27.65	39.78	21.75
3	60	67.43	34.27	46.49	29.54
4	80	68.89	48.54	57.84	37.49
5	100	74.42	53.32	63.24	49.36
IC 50		24.63	90.36	65.65	106.50

4. CONCLUSION

The presence of a significant amount of flavonoid, alkaloid, and phenolic content, as well as significant quantities of secondary metabolites in the rhizomes of the plant investigated here, suggests that the plant might be a source of effective medications. The presence of phytoconstituents in significant quantities may help to recognise the plant's potential pharmacological value in disease management. Plants have medical value because they contain chemical compounds that have a specific physiological effect on the human body. It also validates the plant's folkloric medical usage and claims regarding its therapeutic properties as a cure-all. We recommend that the bioactive chemicals from the rhizomes of K. galanga, K. parviflora, and K. pulchra be isolated, purified, and characterised further in order to develop viable chemotherapeutic drugs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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