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Comparative Study of Phyto-chemical Constituents and Total phenolic, flavonoid content in the extracts of three medicinal plants of Zingiberaceae

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ABSTRACT The rhizomes of the Zingiberaceae family are a vegetable widely used in many Asian countries and their medicinal functions have been broadly discussed and accepted in many traditional recipes. In this study, three medicinal plants of Zingiberaceae family were collected and analysed for their functional properties. The methanolic extraction of three medicinal plant species showed the positive results for the presence of phytochemicals i.e. alkaloids, proteins, carbohydrates, phenols and tannins, flavonoids, saponin, glycosides, steroid and terpenoids. Methanolic extracts of the plants were also analysed for their total phenol compounds and total flavonoid content. The results showed that the total phenol compounds of *Curcuma caesia* was found to be highest among the three medicinal plants of Zingiberaceae and its value is 62 ± 0.2 mg/g whereas the total phenolic content of *Hedychium coronarium* and *Curcuma angustifolia* were found to be 26.22 ± 0.5 mg/g and 20.05 ± 0.5 mg/g respectively. Total flavonoid contents among the three medicinal plants of Zingiberaceae was found to be highest in *Curcuma caesia* and its value was found to be 30±0.007 mg/g.

KEYWORDS : Curcuma caesia, Hedychium coronarium, Curcuma angustifolia

Introduction

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root etc. The beneficial medicinal effects of plant materials typically result from the combinations of these secondary products (Tonthubthimthong et al)[1]. *Curcuma caesia* is commonly known as black turmeric which is an erect rhizomatous herb with large leaves found throughout the Himalayan region, north-east and central India. The paste of rhizome is used traditionally for the treatment of leucoderma, asthma, tumour, piles etc. Essential oil of *C. Caesia* has been known for its antifungal activity. It is Fresh rhizomes are aromatic with intense camphoraceous odour and are applied externally to sprain and bruises. It rhizomes is used for migraine, 2-4 drops of fresh juice is poured in nose. For longevity, impotence, infertility, irregular menstrual flow, a spoonful powder from dried rhizomes is mixed with a spoonful of honey or a cup of milk is taken twice a day. For gastric troubles, a fresh piece of rhizome is chewed (Sahu and Saxena)[2].

Curcuma angustifolia is commonly known as East Indian arrowroot. It is nutritive and used as agreeable, non-irritating diet in certain chronic diseases, convalescence in fever, in irritation of alimentary canal, pulmonary organs and also used in consumption, excessive thirst, jaundice and kidney disorder. Rhizomes are used in inflammation, bone fracture, intestinal diseases by some tribal of Madhya Pradesh and Chhattisgarh states of India. These non- conventional species of *Curcuma* produce starchy rhizomes which are used as remedies for infections, inflammations, gastric and skin disorders but have not been evaluated scientifically for pharmacological activity. The rhizomes of these species are aromatic. Medicinal uses of rhizome arise from the bioactive components. Bioactive components are responsible for antioxidative and anti-inflammatory properties, wound-healing, hypoglycemia, anticoagulant, antimicrobial activities. All most all species of *Curcuma* contains antioxidant activity, the pharmacological effects and prospectus for future clinical use had been tried so far (Dhal et al)[3].

The Zingiberaceae plant *Hedychium coronarium* Koen., which has many common names including butterfly ginger, butterfly lily, cinnamon jasmine, garland flower and ginger lily is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries and so on. The rhizome of *H. coronarium* ("Tuqianghuo"in Chinese) has been used for the treatment of headache, diabetes, contusion inflammation and sharp pain due to rheumatism in Chinese traditional medicine, while it is also used as a febrifuge, tonic, excitant and anti-rheumatic in the Ayurvedic system of traditional Indian medicine (Jain et al)[4]. It has been reported that its rhizomes are used for the treatment of diabetes, tonsillitis, infected nostrils, tumour and fever.

Materials and method

Collection and preparation of plant extract: -The collected aromatic plant species were washed in tap water and then rinsed in distilled water. They were cut into small pieces, shaded, air dried for 7 days and finally dried in an oven at a temperature of 35 – 40ºC for 2 days. The dried plants were pulverized by using grinder to obtain a powdered form. Rhizomes, roots and leaves were powdered separately. Crude extracts of each plant were prepared by Soxhlet extraction method**.** For the methanolic extraction, each 20g of dried and powdered plant material was uniformly packed into a thimble and extracted with 250 ml of methanol at 65ºC. The process of extraction continues for 24 hours or until the solvent in siphon tube of an extractor become colorless. The extract was taken in the amber bottles and kept in refrigerator for future used.

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using the following standard methods.

1. Test for proteins

Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Xanthoproteic test

The extracts were treated with a few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins.

2. Test for carbohydrates

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed

together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test
Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of carbohydrates.

Molisch's test
Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated sulphuric acid was poured carefully along the side of the test tube. Appearance of violet ring at the interphase indicated the presence of carbohydrates.

Iodine test
Crude extract was mixed with 2ml of iodine solution. A dark blue or purple colouration indicated the presence of carbohydrates.

3. Test for phenols and tannins
Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black colouration indicated the presence of phenols and tannins.

4. Test for flavonoids
Shinoda test
Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added dropwise. Pink scarlet colour appeared after few minutes indicated the presence of flavonoids.

Alkaline reagent test
Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of dilute acetic acid indicated the presence of flavonoids.

5. Test for saponins
Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as indication of saponins.

6. Test for glycosides
Liebermann's test
Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated sulphuric acid was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, ie glycone portion of glycoside.

Keller-kilani test
Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

7. Test for steroid
Salkowski's test
Extract was treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids.

8. Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

9. Test for alkaloids
Hager's test
Extract was dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colour precipitate.

Quantitative phytochemical analysis
Determination of total phenolic content
Total phenol content was estimated using Folin-Ciocalteau reagent (McDonald et al)[5]. To 1 ml of each extract (100µg/ml) in methanol, 5 ml of Folin-Ciocalteau reagent (diluted ten-fold) and 4 ml (75 g/litre) of Na_2CO_3 were added. The mixture was allowed to stand at 20º C for 30 minutes and absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100 µg/ml methanolic gallic acid solutions were used as standard for calibration curve. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass) which is a common referernce compound.

Total flavonoids determination
Aluminium chloride colorimetric method was used for flavonoids determination (Chang et al)[6]. Each plant extract (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Results
The phytochemical characteristics of three medicinal plants tested were given in the Table 1. The results revealed the presence of medically active compounds in the three plants studied. From the table it could be seen that proteins, carbohydrates, phenols and tannins, flavonoids, saponins, glycosides, steroid, terpenoids and alkaloids were present in all the three plants.

The total phenolic content of *Curcuma caesia* was found to be highest among the three medicinal plants of Zingiberaceae and its value is 62.00±0.2 mg/g (Table 2) whereas the total phenolic content of *Hedychium coronarium* and *Curcuma angustifolia* were found to be 26.22±0.5 mg/g and 20.05±0.5 mg/g respectively. Total flavonoid contents among the three medicinal plants of Zingiberaceae was found to be highest in *Curcuma caesia* and its value was found to be 30.00±0.007 mg/g. Total flavonoid content in *Hedychium coronarium* and *Curcuma angustifolia* was found to be 27.00±0.001 and 28.00±0.006 respectively. Discussion

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra)[7]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al)[8]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflamatiom, antiatherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han et al)[9]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc (Ali et al)[10]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesised by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. They are also effective antioxidant and show strong anticancer activities (Okwu)[11].

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties in their cytotoxicity (Yadav and Agarwala)[12]. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports ((Nyarko and Addy)[13]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable

reservoir of bioactive compounds of substantial medicinal merit.

Conclusion

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals that contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs.

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Table 1: Comparative analysis of phytochemical constituents in the three medicinal plants of Zingiberaceae

Chemical constituents	Chemical tests	Methanolic extract of *Curcuma caesia*	Methanolic extract of *Hedychium coronarium*	Methanolic extract of Curcuma angustifolia
Alkaloids	Hager's test	+	+	+
Protiens	Millon's test	+	+	+
	Ninhydrin test	+	+	+
	Xanthoproteic test	+	+	+
Carbohydrates	Fehling's test	+	+	+
	Benedict's test	+	+	+
	Molisch's test	+	+	+
	Iodine test	+	+	+
Phenols and tannins		+	+	+
Flavonoids	Shinoda test	+	+	+
	Alkaline reagent test	+	+	+
Saponins		+	+	+
Glycosides	Liebermann's test	+	+	+
	Ferric chloride test	+	+	+
	Keller-kilani test	+	+	+
Steroid	Salkowski's test	+	+	+
Terpenoids		+	+	+

Key: + = present

Table 2: Total phenolic content and flavonoid content of three medicinal plants of Zingiberaceae family

Medicinal plants	Total phenolic content (mg/g)	Total flavonoid content (mg/g)
Curcuma caesia	62.00 ± 0.2	30.00 ± 0.007
Hedychium coronarium	26.22 ± 0.5	27.00 ± 0.001
Curcuma angustifolia	20.05 ± 0.5	28.00 ± 0.006

References

1. Tonthubthimthong P., Chuaprasert S., Douglas P. and Luewisuttichat W. 2001- Supercritical CO2 extraction of nimbin from neem seeds an experimental study. Journal of Food Engineering, 47: 289-293.
2. Sahu R. and Saxena J. 2014- In vitro study of antioxidant activity of conventional and non-conventional species of Curcuma. World Journal of Pharmacy and Pharmaceutical Sciences, 3(7): 934-939.
3. Dhal Y., Deo B. and Sahu R.K. 2012-Comparative antioxidant activity of non-enzymatic and enzymatic extracts of Curcuma zedoaria, Curcuma angustifolia and Curcuma caesia. International Journal of Plant, Animal and Environmental Sciences, 2 (4): 232-239.
4. Jain S.K., Fernandes V.F., Lata S., Ayub A. 1995- Indo-Amazonian ethnobotanic connections-Similar uses of some common plants. Ethnobotany, 7:29-37.
5. McDonald S., Prenzler P.D., Antolovich M., Robards K. 2001- Food Chem. 73: 73-84.
6. Chang C., Yang M., Wen H., Chem J. 2002- Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.
7. Sofowra A. 1993- Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria, pp.191-289.
8. Singh R., Singh S.K., Arora S. 2007- Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. Fod Chem. Toxicol., 45: 1216-1223.
9. Han X., Shen T., Lou H. 2007- Dietry polyphenols and their biological significance. Int. J. Mol. Sci. : 950-988.
10. Ali S.S., Kasoju N., Luthra A., Singh A., Sharanabasava H., Sahuand A., Bora U. 2008- Indian medicinal herbs as source of antioxidants. Food Res. Int., 41: 1-15.
11. Okwu D.E. 2004-Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J. Sustain. Agric. Environ., 6(1): 30-37.
12. Yadav R.N.S., Agarwala M. 2011- Phytochemical analysis of some medicinal plants. Journal of phytology, 3(12): 10-14.
13. Nyarko A.A., Addy M.E. 1990- Effects of aqueous extract of Adenia cissampeloides on blood pressure and serum analyte of hypertensive patients. Phytotherapy Res., 4(1): 25-28.