ISSN: 22313788 (Print)
ISSN: 23214376 (Online)
Journal of PharmaSciTech
2013; 2(2):81-86
Research Article

Pharmacognostic and Phytochemical Evaluation of the Rhizomes of *Curcuma longa* Linn.

Nilanjana Deb*, Purba Majumdar, Ajoy Kumar Ghosh

Gupta College of Technological Sciences, Ashram More, G.T. Road, Asansol-713301, West Bengal, India
Address for correspondence: neeludeb@yahoo.co.in; Tel.: +91-9933867972

Abstract

In ethno medicinal practices, the traditional healers use the genus Curcuma for the treatment of various ailments Turmeric is a spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (Zingiberaceae). Rhizomes are horizontal underground stems that send out shoots as well as roots. Various parameters such as morphology, phytochemical profiles of the entire parts of the plant were studied and the salient diagnostic features are documented. Major chemical constituents, extractive values, physicochemical constants, and other features have also been recorded. Here, important chemicals like alkaloids, flavonoids, and amino acids were identified and isolated.

Keywords: *Curcuma longa,* pharmacognostic standardization, phytochemical screening, CNS depressant activity

Introduction

Use of plant products is increasing in many segment of the population.[1]At present; thousands of plant metabolites are being successfully used for the treatment of variety of diseases. According to an estimate, 80% of the world's population relied upon plants for their medication.[2] The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products.[3] Turmeric is one of most essential spices all over the world with a long and distinguished human use particularly in the Eastern civilization.[4] In India, *Curcuma longa* has been in use as a culinary ingredient since 3000 BC. It is used as a food colouring for curry and as a preservative for food. As a medicine it is used to treat a wide variety of ailments including stomach ache, skin problems, muscular problems and arthritis.

Curcuma longa has also been used as a clothing dye and as a cosmetic. Indians are thought to consume between 80 and 200 mg per day of *Curcuma longa* extract. India as a whole consumes 480,000 tons of turmeric annually. The origin of the plant is not certain, but it is thought to be originated from south eastern Asia, most probably from India. The plant is cultivated in all parts of India.[5] India produces most of the world supply [6], but turmeric is cultivated also in southern China, Taiwan, Japan, Burma, and Indonesia [7]as well as throughout the African continent [8]. The commercially available material (i.e. turmeric powder) in Europe is obtained mainly from India and somewhat from other south eastern Asian countries.[9] It is generally recognized as safe by the FDA of the United States. Availability: in capsule form as a health supplement and as the culinary spice Turmeric. The effective use of *Curcuma longa* Linn. is well known since a long time; it is laxative, anthelmintic, and vulnerary, besides this it is used in blood disorders, leukoderma, scabies, small-pox, and sprains. The inner part of rhizome is bluish black in colour and emits a characteristic sweet smell. The "turkomans" (Turkish people) employ these roots as a rubeficient to rub the body after taking a Turkish bath. In Bengal, it is used in the fresh state–turmeric.[10] The plant is regarded as very auspicious and it is often used in India for various magic remedies. The rhizomes of the herb are often used by the Baiga, Sahariya, Agariya, Gond, Korku, and other tribal community of Mandla, Balaghat, and Chhindwara. The powder of herb is used by tribal women as a face-pack during their engagement and marriage period.[11] Due to its increasing demand and overexploitation without ensuring its regeneration, the plant has recently been categorized as an endangered species, the plant is also having some amount of antifungal protein against drug-resistant *Candida albicans*.[12] The present work has been performed to study the phytochemical parameters, which could serve as a measure of authentication and quality establish the various Pharmacognostic and control for commercial samples of crude drug. The detailed macroscopy of various parts of the plant has also been studied and documented. Its uses in traditional medicine are reviewed in Table 1.

Materials and Methods

Materials

The reagents such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, alpha naphthol, Conc. HCl, Sodium hydroxide, Chloroform were procured from Qualigens,

India. Other reagents like Pyridine, Zinc dust was procured from Merck, India and that of magnesium ribbon from Loba Chemie Pvt. Ltd., India. Diazepam was obtained as a gift from Ranbaxy Ltd., India). All other chemicals and reagents were used as received.

Identification of sample

Curcuma longa is a member of the ginger family (Zingiberaceae).[13] Rhizomes are horizontal underground stems that send out shoots as well as roots. *Curcuma longa* rhizomes were purchased from the local market of Asansol, West Bengal, India and authenticated by the herbarium of Botanical Survey of India, Botanic garden, Shibpur, Howrah, bearing the reference number- CNH/II (272)/ 2008/Tech II/315.

Pharmacognostic evaluation

Total ash value[14]

It depicts the total amount of material produced after the complete incineration of the ground drug above 400°C to remove all the carbon atoms. 2g of powdered drug was weighed and placed in the crucible and heated at about 400°C. The crucible was cooled and the % of the total ash with reference to the air-dried sample of the crude drug was calculated.

Acid insoluble ash[14]

Total ash obtained was dissolved in 1N HCl solution and heated for 5 min. The insoluble matter was filtered in whatman filter paper; the filter paper was further dried at 70°C and then cooled. The residue was weighed and the percentage of insoluble ash of the crude drug w.r.t. the air dried sample of crude drug was calculated.

Water soluble ash[14]

To the total ash crucible, 25ml double distilled water was added and boiled for about 5min. Insoluble matter was collected on an ash less filter paper in a crucible, washed with hot water and ignited for about 15min above 45°C. The weight of the residue is subtracted from the weight of the total ash. Content of water soluble ash in mg/g of the air dried material was calculated.

Determination of solvent extractive value[14]

2g of the air- dried coarsely powdered drug was macerated with 100ml of different solvents in closed flasks for 24h, and are shaken frequently. The solvent was filtered and the filtrate was weighed in a Petri dish. The dish was hen evaporated on a water bath and then dried in an oven at 100°C. The dish was cooled and extractible value was calculated as % (w/w) with reference to air dried drug.

Loss on drying (LOD)

LOD is the loss in weight in % (w/w) resulting from water and volatile matter of any kind that can be driven off under specified conditions. 1g sample is transferred to a shallow bottle and weighed. Sample was distributed evenly and dried in a hot air oven at 105°C for 1h with the stopper open. After 1h, the stopper was closed and cooled at room temperature and the bottle was weighed.

Preparation of the extract

The rhizomes of *Curcuma longa* were collected and dried in sun for 3 days, cut into small pieces and again dried. The upper bark of the rhizome was removed to obtain the fresh rhizome. The dried rhizome was then grinded to obtain a fine powder. The powder was again dried and was ready for use.

Ethanolic extraction

The grinded powder was extracted with 500ml of dehydrated ethanol and 1000ml double distilled water respectively by Soxhlation for 72h. The extract was concentrated at temperature <45°C. The residue was dried and refrigerated.

Aqueous extraction

The grinded powder was then extracted with 1000ml double distilled water containing 3-4 drops of chloroform for 48h. The extract was then concentrated at temperature less than 45°C.The residue was then dried and refrigerated.

Qualitative chemical tests

Test for alkaloids[15]

(a) **Mayer's test:** To 2ml test solution, 2N HCl was added. The aqueous layer formed was decanted and Mayer's reagent (Qualigens, India) was added to it. A cream coloured precipitate indicates the presence of alkaloids.

(b) **Dragendroff's test:** To 2ml test solution, and Dragendroff's reagent (Qualigens, India) was added to it. A reddish brown precipitate indicates the presence of alkaloids.

(c) **Wagner's test:** To 2ml test solution, and Wagner's reagent (Qualigens, India) was added to it. A reddish brown precipitate indicates the presence of alkaloids.

(d) **Hager's test:** To 2ml test solution, and Hager's reagent (Qualigens, India) was added to it. A yellow coloured precipitate indicates the presence of alkaloids.

Test for glycosides[15]

(a) To 2ml test solution, equal quantity of Fehling's solution A and B was added and solution was heated. A brick red precipitate indicates the presence of glycosides.

(b) Legal's test: To 2ml test solution, pyridine (Merck, India) and alkaline sodium nitroprusside was added to obtain a blood red colour.

Test for flavonoids[15]

(a) **Shinoda test:** To 2ml test solution, few fragments of Magnesium ribbon (Loba Chemie, India) were added and to it conc. H2SO4 was added drop wise. Pink scarlet or crimson red colour appears.

(b) **Zinc chloride reduction test:** To 2ml test solution, a mixture of zinc dust (Merck, India) and conc. HCl (Qualigens, India) was added. A red colour is obtained after few minutes.

(c) **Alkaline reagent test:** To 2ml test solution, sodium hydroxide (Qualigens, India) solution was added to give a yellow or red colour.

Test for tannins

(a) **Gelatin test:** To 2ml test solution, 1% Gelatin solution containing 10% sodium chloride was added to obtain a white precipitate.

(b) **Ferric chloride test:** To 2ml test solution, ferric chloride was added to give a blue green colour.

Test for proteins and amino acids

(a) **Millon's test:** To 2ml test solution, Millon's reagent is added which gives a white precipitate, which on heating changes to red.

(b) **Ninhydrin test:** To 2ml test solution, ninhydrin solution was added and the solution was boiled. Amino acids and proteins when boiled with 0.2% ninhydrin reagent show a violet colour.

Test for fats and fixed oils

(a) **Stain test:** Small amount of the extract was pressed between two filter papers; the stain on the filter paper indicates the presence of fixed oils.

(b) **Saponification test:** Few drops of 0.5N alcoholic potassium hydroxide was added in small quantity to the extract solution with a drop of phenolphthalein and heated on a water bath for 1-2h. The formation of soap or partial neutralization for the alkali indicates the presence of fats and fixed oils.

Test for Sterols[16]

Liebermann-Burchard test: To the test solution, 3-4 drops of acetic anhydride was added, the solution was boiled cooled and conc. Sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids

Test for triterpenoids[17]

Salkowski test: To the test solution 2ml chloroform (Qualigens, India) was added with few drops of conc. Sulphuric acid (3ml), and shaken well. Appearance of reddish brown colour at lower layer indicates presence of steroids and that of yellow colour shows the presence of triterpenoids.

CNS depressant activity of the ethanol and aqueous extracts of *Curcuma longa*

Two standard neuropharmacological experimental methods viz. motor coordination and loco-motor methods were employed to determine the CNS depressant activity.

Animals

Swiss albino mice weighing 25-30 g were used. They were caged in a room under Standard laboratory conditions (temperature 23±1°C, relative humidity 55% ±5%). The animals were fed with standard pellet diet and water *ad libitum*. The animals were kept under alternate cycle of 12h of darkness and light. The animals were acclimatized to the laboratory. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during the day. The ethical committee of the institute approved the protocol of the study.

Motor coordination[18]

Digital rotarod apparatus (Biological Museum, Agra, India) was used to evaluate the muscle relaxing and sedative effects in the extract and vehicle treated mice. The animals were trained to remain for 3 min on the rod rotating at a speed of 25 rpm. Only animals performing up to the required parameter were included in the test and divided into six groups. Group I served as control and received only vehicle. Group II received reference standard diazepam (at a dose of 2mg/kg) i.p. 30 min before the test. Group III and IV received the aqueous extract of *Curcuma longa* rhizomes (200mg/kg and 300 mg/kg, respectively) Groups V and VI were treated intraperitoneally with the ethanol extract of *Curcuma longa* (200mg/kg and 300 mg/kg, respectively) All animals were subsequently assessed for their performance on the rotarod after 30min. The fall off time from the rod was noted for each animal.

Locomotor activity[19]

Locomotor activity was recorded with a using a digital activity cage Actophotometer (Techno world, Delhi India). The animals were kept at fasting condition for 18h. The animals were then divided into six groups (n = 6). Each mouse was individually placed in the actophotometer for 5 min. Six India groups of animals were each treated (i.p) with vehicle and extract. Basal reaction time was noted before and 30 min after the administration of treatment. A count is recorded when the beam of light falling on the photocell of actophotometer is cut off by mice. Group II received reference standard Diazepam at a dose of 3 mg/kg (i.p.) 30min before the test. Mean change in the locomotor activity was recorded for each group.

Results and Discussion

We have studied and evaluated the different Pharmacognostic parameters in order to standardize the drug. The results of Pharmacognostic parameters such as ash value, extractive value, LOD have been cited in Table 2. The Ethanolic extract of *Curcuma longa* rhizomes indicated the presence of flavonoids and amino acids (Table 3); whereas the aqueous extract was found to contain alkaloid in addition to flavonoids, amino acids (Table 3).

In this work, the effects of aqueous and ethanol extracts of *Curcuma longa* rhizomes were studied in two neuropharmacological models. The Aqueous extract of *Curcuma*

longa (AECL) at a dose of 300mg/kg body weight showed depression in the motor coordination activity coordination activity upto 85.26% by the Rota rod apparatus (Table 4). It was also found that AECL at a dose of 300mg/kg body weight showed significant decrease in the locomotor activity upto 64.52% compared to the EECL which at the same dose showed a decrease upto 51.45% (Table 5). The results of the study provided evidence that the aqueous extracts of *Curcuma longa* rhizomes (AECL 300mg/kg) possess a spectrum of CNS activity (Table 4-5). Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity.[20] The effect on locomotor activity works to the advantage of the plant compounds and requires preformulation studies for development of a potential dosage form. Thus from Table 4 - 5, it can be concluded that the aqueous extract (AECL), has greater CNS depression activity in comparison with the Ethanolic extract (EECL) .

Table 1. Various medicinal use of the plant *Curcuma longa* Linn.

Species	Family	Uses
Curcuma longa	Zingiberaceae	Fresh turmeric rhizome, ground with cow milk and castor oil is applied externally to treat paronychia. Dried rhizome is used on fresh wounds as a counterirritant on insect stings to facilitate the scabbing process in chickenpox and smallpox Turmeric powder mixed with the juice of Aloe Vera is used externally to treat wounds. The powder mixed with Murraya paniculata paste is used externally for fractured bones. Water extract of dried root mixed with *Alangium Salvifolium* powder is used externally for wounds and vaginal discharge.

Table 2. Pharmacognostic study of the plant *Curcuma longa* Linn.

Parameters of evaluation	Values in percentages
Ash Value	
Total ash value	13.30
Acid insoluble ash value	1.00
Water soluble ash value	4.20
Extractive value	
Alcohol soluble extractive value	18.45
Water soluble extractive value	22.00
Loss on drying	4.14

Table 3. Phytochemical tests for identification of chemical constituents in ethanolic and aqueous extract of the rhizomes of *Curcuma longa* Linn.

Phytochemical constituents	Name of the tests	Ethanolic extract	Aqueous extract
Alkaloids	Mayer's test	Negative	Negative
	Dragendroff's test	Negative	Positive
	Wagner's test	Negative	Positive
	Hager's test	Negative	Positive

Phytochemical constituents	Name of the tests	Ethanolic extract	Aqueous extract
Glycosides	Fehling's test	Negative	Negative
	Legal's test	Negative	Negative
Flavonoids	Shinoda test	Negative	Positive
	Zinc chloride reduction test	Positive	Positive
	Alkaline reagent test	Positive	Positive
Tannins	Gelatin test	Negative	Negative
	Ferric chloride test	Negative	Negative
Proteins	Millon's test	Negative	Negative
Amino acids	Ninhydrin test	Positive	Positive
Fats and fixed oils	Stain test	Negative	Negative
	Saponification test	Negative	Negative
Sterols	Leibermann- Burchard test	Negative	Negative
Terpenoids	Salkowski test	Negative	Negative

Table 4. Motor coordination activity of the rhizomes of *Curcuma longa* Linn. by Rota rod apparatus

Sl.No	Treatment	Dose (mg/kg)	Fall off time (sec)		% Decrease in time
			Before drug	After drug	
1	Control	10ml	545±51.67	564.1±46.84	-
2	Diazepam	3	534.5±66.07	48.8±4.92**	90.86
3	AECL	200	495.16±72.22	89±69.93**	81.88
4	AECL	300	525.66±60.18	78±15.84**	85.26
5	EECL	200	526.5±81.37	201.83±38.7	61.66
6	EECL	300	548.33±43.08	240±211.33*	51.81

Table 5. Locomotor activity of the rhizomes of *Curcuma longa* Linn. by Actophotometer

Sl.No	Treatment	Dose (mg/kg)	Locomotor activity scores in 10 min		% Decrease in time
			Before drug	After drug	
1	Control	10ml	307.52±19.50	301.0±9.44	-
2	Diazepam	3	314.83±22.48	49.67±14.6*	84.22
3	AECL	200	297.50±20.09	121.30±9.77*	59.22

Sl.No	Treatment	Dose (mg/kg)	Locomotor activity scores in 10 min		% Decrease in time
			Before drug	After drug	
4	AECL	300	330.30±23.59	117.16±16.55*	64.52
5	EECL	200	317.32±30.80	241.0±33.89*	24.05
6	EECL	300	320.33±30.80	155.5±11.80*	51.45

Conclusion

The plant *Curcuma longa* is an ayurvedic plant. Experimental data from the international literature, as reviewed herein, indicates plenty of information about the use of turmeric as a spice and apart from its multiple medicinal uses. After performing the work, it was found that, the aqueous extract of *Curcuma longa* contains alkaloids, amino acids and flavonoids. On the basis of results obtained from this investigation, we can conclude that the extracts of *Curcuma longa* have neuropharmacological activity as evident by significant reduction in locomotor activity and muscle coordination. It is logical to suggest that it may be useful as CNS depressant agent in clinical conditions. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

Acknowledgement

The authors are grateful to Professor Debesh Chandra Majumdar, Chairman, Gupta College of Technological Sciences, Asansol, West Bengal, India and to the college for supporting the project, and providing assistance.

References

1. Eisenberg DM, Kessler RC, Foster C. Unconventional medicine in the United States: prevalence, costs and patterns of use. New Eng J Med, 1993; 328: 246-252.

2. Akerele O. Nature's medicinal bounty: don't throw it away. World Health Forum 1993; 14: 390-395.

3. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. New York: John Willey Sons Ltd, 1982:6.

4. Ravindran PN. Turmeric: The golden spice of life. In: Ravindran PN, Nirmal Babu K,Sivaraman K, eds. Turmeric: The Genus Curcuma. USA: CRC Press, Boca Raton, 2007: 1-14.

5. Kapoor LD. Handbook of Ayurvedic Medicinal Plants. USA: CRC Press, Boca Raton, 2000.

6. Leung AY, Foster S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. 2nd ed. New York: John Wiley & Sons, 1996.

7. Yen K-Y. The Illustrated Chinese Materia Medica: Crude and Prepared. Taiwan: SMC Publishing, 1992.

8. Iwu MM. Handbook of African Medicinal Plants. USA: CRC Press, 1993.

9.Murugananthi D, Selvam S, Raveendaran N, Meena ST. A study on the direction of trade in the Indian turmeric exports: Markov chain approach. IUP J Agr Econ 2008;4:20-25.

10. Nadkarni KM. Indian Material Medica, Volume 1. Bombay: Popular Prakashan;1976: 414.

11. Chourasia V. Self medication of Baiga tribal's, Aayushman. 2006: 49.

12. Kirtikar KR, Basu RD. Indian Medicinal Plants. Dehradun: International book distributors, 1987: 2418–2426.

13. Dobelis IN. Magic and Medicine of Plants. Pleasantville, NY: Reader's Digest Association, Inc. 1986.

14. Harborne JB. Phytochemical methods. London: Chapman and Hall, Ltd, 1973:49-188.

15. Mukherjee PK. Quality Control of Herbal drugs. New Delhi: Business Horizons, 2002: 186-191.

16. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Nigeria: Spectrum Books Ltd, 1993:289.

17. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Pune, India: Nirali Prakashan, 2007:593-598.

18. Achliya GS, Wadodkar SG, Dorle AK. Neuropharmacological actions of Panchagavya formulation containing Emblica officinalis Gaerth and Glycyrrhiza glabra Linn in mice. Indian J Exp Biol 2004; 42: 499-503.

19. Turner RA. Depressants of the central nervous system. In: Turner RA,ed. Screening Methods in Pharmacology. New York: Academic press, 1972: 78-88.

20. Lowry CA, Johnson PL, Hay Schmidt A, Mikkelsen J, Shekhar A. Modulation of anxiety circuits by serotonergic systems. Stress 2005; 8: 233-246.