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Research Article

ANALGESIC AND ANTIMICROBIAL ACTIVITIES OF *CURCUMA ZEDOARIA*

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ABSTRACT

This study describes the biological activity of the dried leaves, stem and rhizomes of *Curcuma zedoaria* belonging to the family Zingiberaceae. The dried powders of the plant were extracted with organic solvents- methanol and pet ether sequentially by the maceration process. The crude extract of methanol and pet ether were then investigated for analgesic and antimicrobial activity. At an oral dose of 250 mg/kg of body weight the crude extracts of methanol and pet ether were studied for analgesic property by using acetic acid induced writhing effect method. The methanolic rhizome extracts had mild analgesic property (with writhing inhibition of 66.67%). Pet ether extracts of the rhizome, leave and stem had shown moderate analgesic property (with writhing inhibition of 70.24 %, 75 %, 71.43 % respectively), while pet ether extracts of the leaves showed significant analgesic property (with writhing inhibition of 91.67 %). The crude extracts of methanol and pet ether were screened for antimicrobial activity against gram positive and gram negative bacteria and fungi using disc diffusion method. The results obtained were compared with that of standard drug kanamycin. The stem extract showed mild sensitivity to several gram positive, gram negative bacteria and fungi (zone of inhibition 7 mm). The pet ether leaf extract and methanolic leaf extract also showed mild sensitivity to several gram positive, gram negative bacteria and fungi (zone of inhibition 10-12 mm and 11-12 mm respectively). Pet ether rhizome extract also showed highly sensitivity to several gram positive, gram negative bacteria and fungi (zone of inhibition 11-14 mm). The methanolic rhizome extracts showed significant sensitivity to several gram positive, gram negative bacteria and fungi (zone of inhibition 13-15 mm).

Keywords: *Curcuma zedoaria, Zingiberaceae,* Analgesic activity, Acetic acid, Antimicrobial activity, Disc diffusion.

INTRODUCTION

Curcumazedoaria Rosc, also known as white turmeric, zedoaria or gajutsu[1], is a perennial rhizomatous herb that belongs to the Zingiberaceae family. The plant is indigenous to Bangladesh, Sri Lanka and India, and is also widely cultivated in China, Japan, Brazil, Nepal and Thailand. In India it is known by its several vernacular names, the most commonly used ones being Krachura (Sanskrit), Gandamatsi (Hindi) and Sutha (Bengali)[2], It is used traditionally for the treatment of menstrual disorders, dyspepsia, vomiting[3] and for cancer[4], Rural people use the rhizome for its rubefacient, carminative, expectorant, demulcent, diuretic and stimulant properties while the root is used in the treatment of flatulence, dyspepsia, cold, cough and fever[1], Zedoaria is a herbaceous and rhizomatous perennial plant composed of an upright pseudostem, a corm and underground cylindrical branches or rhizomes and fleshy roots. Some roots develop terminal storage structures (rounded to elongated tuber-like roots called t-roots). From March to April the axillary buds of the corm and apical buds of the third-order rhizomes emerge above the ground as inflorescences. This basal flower spike, which grows about 30cm tall, appears just before the foliage. On the node closest to the flower spike, a vegetative shoot always develops. No additional floral buds sprout but more vegetative shoots develop. New branches start to develop on corms of recently formed aerial shoots. By autumn, the above-ground foliage dies back. From November to December storage roots are formed, having a high (>70%) carbohydrate content[5].

Fig. 1: *Curcuma zedoaria*

Traditional Uses

Curcuma zedoaria is a well known traditional plant that is also used in Ayurveda. Its use in the Indian traditional folk medicine is also well documented. Table 1 indicates the use of different parts of *Curcuma zedoaria* in traditional systems of medicine[2, 3, 6, 7].

Table 1: Traditional uses of *Curcuma zedoaria*

Plant parts	Traditional uses
Oil of rhizome	Stomachic, emmenagogic, vomiting, menstrual haematometra[3]
Fresh roots	Treatment of leucorrhoeal discharge[6]
Tuber juice	Treatment of worms in children[2]
Powdered rhizome	Antiallergant[6]
Leaf juice	Treatment of dropsy[2]
Leaf juice	Treatment of leprosy[7]
Leaf paste	As plasters in lymphangitis, furunculosis[7]

Phytochemistry

Curcuma zedoaria is a rich source of essential oils, starch, curcumin, arabin, gums, etc[2]. Makabe *et al*[8] have isolated more than 10 sesquiterpenes from the rhizome of *Curcuma zedoaria* and were able to structurally characterize 15 such compounds, namely furanodiene, furanodienone, zedorone, curzerenone, curzeone, germacrone, 13-hydroxygermacrone, dihydrocurdione, curcumenone and zedoaronediol.

Phytochemical analysis was carried out by Navarro *et al*[9] using air-dried rhizomes (3kg). The powder was extracted twice with dichloromethane at room temperature for five days, and then with ethyl acetate and methanol, respectively. The extracts were then concentrated under reduced pressure to give the respective fractions. A part of the dichloromethane fraction (50g) was chromatographed using a silica gel column eluted with a mixture of hexane–ethylacetate in increasing polarity. The fraction F1(3.5g), obtained from the above, was rechromatographed over a silica gel column and, when eluted with benzene–acetone (9:1), yielded about 500 mg of compound 1 and 150 mg of compound 2. Spectroscopic data (IR and NMR) confirmed identity of compound 1 as curcumenol and compound 2 was a mixture of phytosterols (especially sitosterol and stigmasterol 2:1).

Christiane *et al* [10] described the seasonal variation of curcumenol and dihydrocurdione, two active terpenoids from different parts (roots, mother rhizome and rugous rhizome) of *Curcuma zedoaria* grown in Brazil. The analysis was carried out by high resolution gas chromatography, using external standards for determination. The results showed that both terpenoids are present in all the parts studied. However, *Curcuma zedoaria* exhibited about three times more terpenoids in the mother rhizome in autumn than in other parts and seasons studied.

A new eudesmane-type sesquiterpene, zedoarofuran, and six new guaiane or secoguaiane-type sesquiterpenes, 4-epicurcumenol, neocurcumenol, gajutsulactones A and B and zedoarolides A and B, were isolated from the aqueous acetone extract of zedoaria rhizome together with 36 known sesquiterpenes and two diarylheptanoids. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence[11]. Two guaiane derivatives were isolated from the rhizomes of *Curcuma zedoaria*. Their structures, zedoalactone A and zedoalactone B, were established by ^{1}H and $^{13}CNMR$ spectroscopic studies and by comparison with closely related compounds[12]. Zedoarol, 13-hydroxygermacrone and curzeone were isolated and structurally elucidated by Shiobara *et al*[13] using *Curcuma zedoaria*.

Three sesquiterpenoids, curcumenone, curcumanolide-A and curcumanolide-B, were isolated from the dried rhizome of *Curcuma zedoaria* by Shiobara *et al*[14] Ethyl para-methoxycinnamate (16) was isolated from the methanolic extract of *Curcuma zedoaria* by chromatography on neutral alumina and silica gel[15]. In the course of searching for biologically active sesquiterpenoids from the Curcuma genus, two sesquiterpenoids were isolated from the rhizome of *Curcuma zedoaria*. Their structures were identified as a-turmerone and b-turmerone. The structural elucidation of these compounds were carried out by comparison of their physical and spectral data with previously reported values[16]. Mau *et al*[17] isolated essential oils from the rhizomes. They isolated a total of 36 compounds but were only able to structurally characterize epicurzerenone and curzerene.

The essential oil obtained by hydrodistillation of the rhizome of *Curcuma zedoaria* native to north-east India has been analyzed by gas chromatography (GC) and gas chromatography–massspectrometry (GC-MS). Thirty seven constituents representing about 87.7% of the total oil have been identified. Curzerenone (22.3%) was the major component, followed by 1,8-cineole (15.9%) and germacrone (9.0%)[18]. The chemical investigation on essential oils of rhizomes of *Curcuma zedoaria*, done by GC and GC-MS, revealed the presence of 1,8-cineole (18.5%), cymene (18.42%), a-phellandrene (14.9%) and b-eudesmol (10.6%)[19].

The essential oil produced by hydrodistillation of *Curcuma zedoaria* leaves was investigated by GC and GC-MS. Twenty-three compounds were identified, accounting for 75% of the oil. The oil of *Curcuma zedoaria* was made up mainly of mono- and sesquiterpenoids, monoterpene hydrocarbons (2.3%), Oxygenated monoterpenes (26%), sesquiterpene hydrocarbons (38%) and oxygenated sesquiterpenes (13.5%). The major constituents of the leaf oil were a-terpinylacetate (8.4%), isoborneol (7%) and dehydrocurdione (9%)[20].

Chemical analysis of the volatile oil from *Curcuma zedoaria* using GC-MS technique revealed the presence of b-tumerone (19.88%), 1, 8-cineole (8.93%) and zingiberene (7.84%) as major constituents[21].

The essential oil of the dried rhizome was isolated using simultaneous steam distillation and solvent extraction and its fractions were prepared by silica gel column chromatography. In total, 36 compounds were identified in the essential oil, including 17 terpenes, 13 alcohols and 6 ketones. Epicurzerenone and curzerene were found in the first and second highest amounts (24.1 and 10.4%) [17].

Curcumin, dihydrocurcumin, tetrahydrodemethoxy-curcumin and tetratetrahydrobisdemethoxycurcumin were isolated together with two bisabolane-type sesquiterpenes from 80% aqueous acetone extract of the rhizome of *Curcuma zedoaria*. Bioassay-directed fractionation of an ethanol extract of *Curcuma zedoaria* led to the isolation of an active curcuminoid, which was identified as demethoxycurcumin by comparison of its ^{1}H and $^{13}CNMR$ spectra with literature data and by direct comparison with synthetic material. Curcumin and bisdemethoxycurcumin were also obtained[22].

The variation of curcuminoids in the ethanolic extract of *Curcuma zedoaria* was measured by using high-performance liquid chromatography (HPLC). The analysis was carried out at 425 nm using a BDS Hypersil C18 column as astationary phase, 0.1% acetic acid aqueous solution and acetonitrile as mobile phase. Ethanolic extracts of *Curcuma zedoaria* rhizomes collected from various parts of Thailand contained curcumin, demethoxycurcumin and bisdemethoxycurcumin in the range of 1.46 ± 0.45 to 5.73 ± 0.11% w/w (average 2.73 ± 1.24% w/w), 3.15 ± 0.15 to 10.98 ± 0.28% w/w (average 7.37 ± 2.71% w/w) and 0.49 ± 0.02 to 2.99 ± 0.20% w/w (average 1.40 ± 0.82% w/w) respectively. The highest average total curcuminoid content in the extracts was found to be 16.83 ± 0.62% w/w while the lowest content was 6.09 ± 1.79%w/w. This information will be useful as a guide for further standardization of *Curcuma zedoaria* extracts for which the content has not been reported elsewhere (Figure 2)[23].

Figure 3 illustrates the structures of a few biologically active compounds that have been isolated from *Curcuma zedoaria*. Table 2 shows the percentage of various phytoconstituents present in *Curcuma zedoaria*.

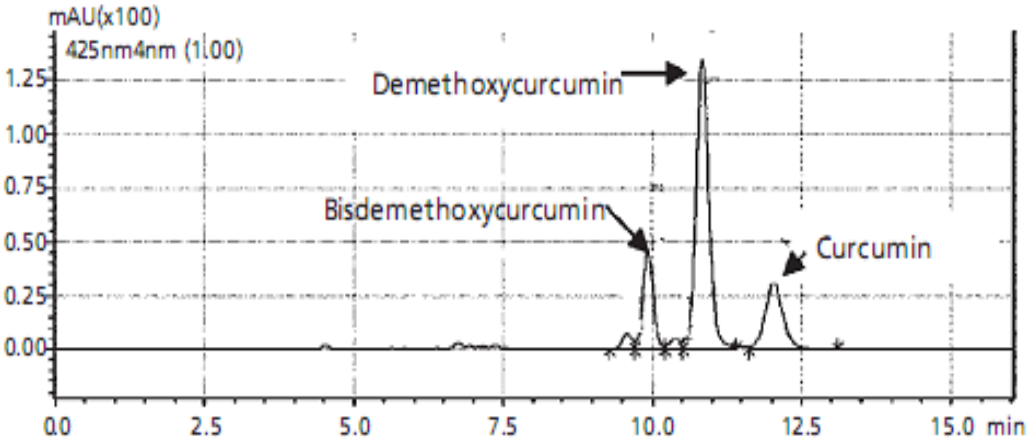

Fig. 2: Chromatogram of 70% ethanolic extract of *Curcuma zedoaria* rhizome.

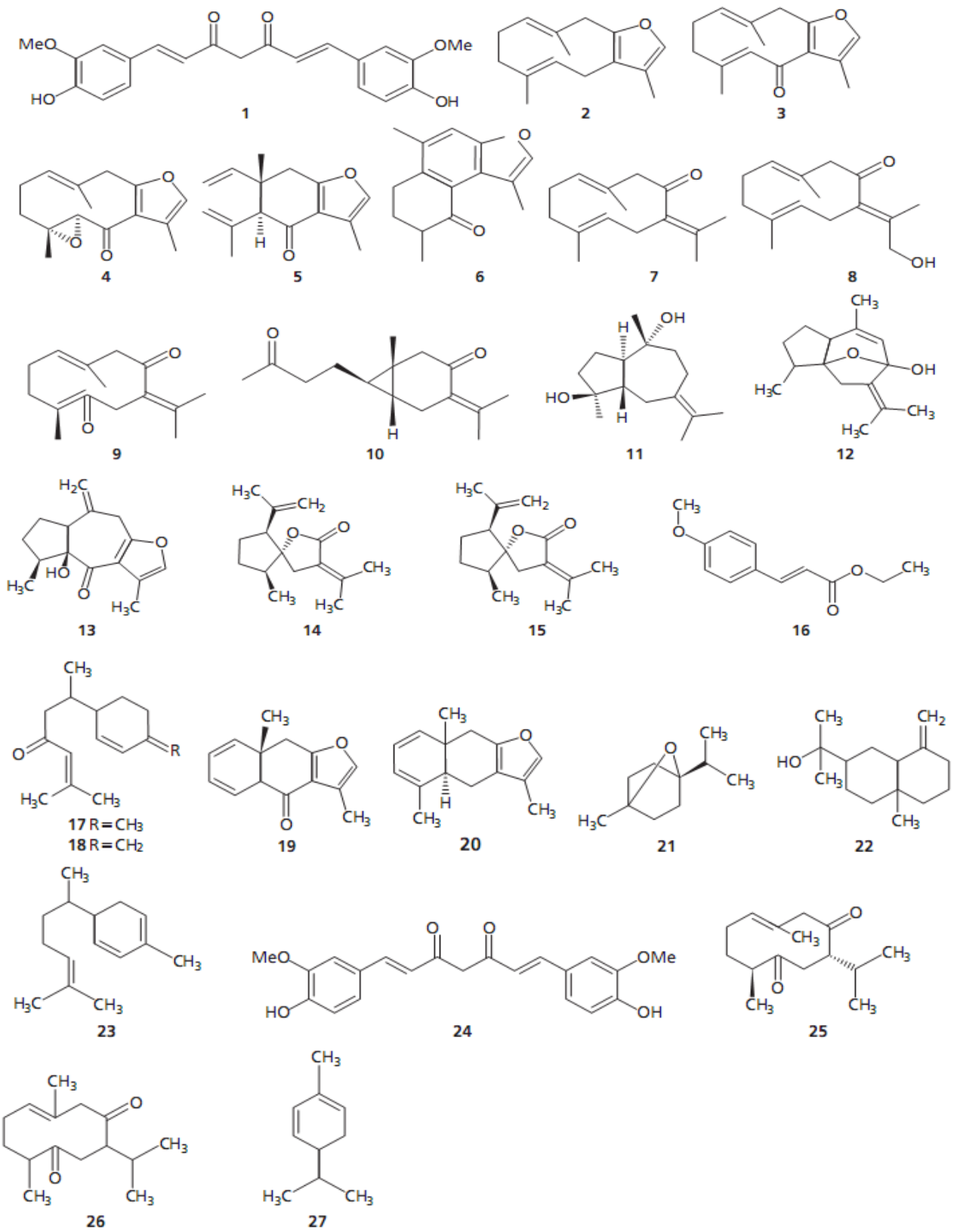

Fig. 3: Structures (listed below) of the biologically active compounds isolated from *Curcuma zedoaria*.

1. Cucrumin, 2. Furanodiene, 3. Furanodienone, 4. Zedorone, 5. Curzerenone, 6. Curzeone, 7. Germacrone, 8. 13-hydroxygermacrone, 9. Dihydrocurdione, 10. Curcumenone, 11. Zedoaronediol, 12. Curcumenol, 13. Zedoarol, 14. Curcumanolide-A, 15. Curcumanolide-B, 16. Ethylpara-methoxycinnamate, 17, 18, β-turmerone, 19. Epicurzerenone, 20. Curzerene, 21. 1, 8-cineole, 22. β -eudesmol, 23. Zingiberene, 24. Dihydrocurcumin, 25. Curdione, 26. Neocurdione, 27. α- phellandrene.

Table 2: Percentage of various phytoconstituents present in *Curcuma zedoaria*.

Source	Active constituents	Percentage(%)
Oil from *Curcuma zedoaria* rhizome[18]	Curzerenone	22.3
	1, 8 Cineole	15.9
	Germacrone	9.0
Oil from *Curcuma zedoaria* rhizome[19]	Cymene	18.42
	α-Phellandrene	14.90
	β -Eudesmol	10.60
Oil from *Curcuma zedoaria* leaves[20]	Monoterpene hydrocarbon	2.3
	Oxygenated monoterpene	26.0
	Sesquiterpene hydrocarbon	38.0
	Oxygenatedsesquiterpene	13.5
	α-Terpinylacetate	8.4
	Isoborneol	7.0
	Dehydrocurdione	9.0
Volatile oil from *Curcuma zedoaria*[17]	Epicurzerenone	24.1
	Curzerene	10.4

MATERIALS AND METHODS

Collection and preparation of plant sample

Reagents and chemicals used

Reagents and chemicals	Source
Diclofenac	Sigma, USA
Acetic acid	Merck, Germany
Tween-80 (as suspending agent)	BDH Chemicals Ltd.
DMSO (as suspending & solubilizing agent)	Merck, Germany
Normal saline solution	Beximco Infusion Ltd.

Equipments used

Equipments	Source
Sterile disposable syringe (1ml, 100 divisions)	CHPL, India
Tuberculin syringe with ball shaped end	Merck, Germany
Electronic and digital balance	Denver Instruments M-220

Fresh *Curcuma zedoaria* was collected from Chittagong hill track region of Bangladesh. It was identified locally. The leaves, stems parts and rhizomes were washed, sliced and then sun dried. Finally the dried leaves, stems and rhizomes were ground to coarse powders by using a grinding machine.

Extraction of the sample

About 170 gm of the powdered rhizome was taken in a clean beaker and soaked in 500 ml of methanol. The beaker with its content was sealed by foil and kept for a period of 9days with occasional shaking. The mixture was then filtered. About 150 gm of the powdered leaves was taken in a clean conical flask and soaked in 500 ml of methanol. The beaker with its content was sealed by foil and kept for a period of 9days with occasional shaking. The mixture was then filtered. About 35 gm of the powdered rhizome was taken in a separate beaker and soaked in 300 ml of methanol. The beaker with its content was sealed by foil and kept for a period of 9days with occasional shaking. The mixture was then filtered. The filtrate of rhizomes, leaves and stem parts thus obtained was kept at room temperature with protective measure from dust and the solvent is allowed to evaporate. The dried crude extract of rhizomes were respectively 5.5 gm, leaves 6.5 gm, stem 3.2gm.

Solvent-solvent partitioning

The solvent-solvent partitioning was done by using the protocol designed by Kupchan and modified by Wagnen *et al.* (1973). The crude extract of rhizomes (5.5) was dissolved in 10 % aqueous Methanol. It was then extracted with pet ether. Same procedure was carried for leaves. The whole partitioning process was carried out through the following schematic representation.

Test with the acetic acid induced writhing method

Navarro *et a.*[9] investigated the analgesic activity of *C. zedoaria* rhizomes grown in Brazil. From the rhizome's hydroalcoholic extract, different fractions (dichloromethane, ethyl acetate, methanol) were prepared and tested for analgesic activity along with curcumenol. Aspirin and dipyrone were used as standard drugs. The activity was investigated using several models of pain in mice, including writhing, formalin and capsaicin. Curcumenol presented promising analgesic effects, being several times more potent than the reference drugs evaluated in the same experimental model.

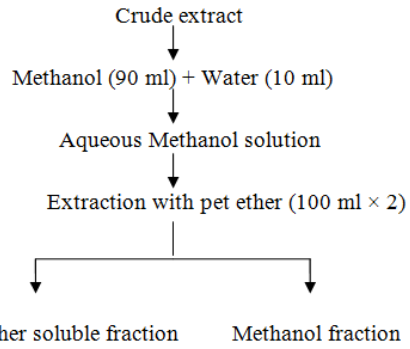

Fig. 4: Schematic representation of the partitioning.

Experimental animal

Swiss-albino mice of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B) were used in the experiment. They were kept in standard environmental condition and fed ICDDR, B formulated rodent food and water.

Experimental design

Twenty four experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV, consisting of 4 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

Method of identification of animals

Each group consisted of four mice. 10% aqueous solution of picric acid was applied to the back of the animals by means of a brush. The back was marked on three areas. A spot on the left side of the back was marked to identify mouse no.1, far right side of the back was applied by brush to designate mouse no.2, spot on the middle field of the back was made to identify mouse no.3 and finally the tail was marked to designate mouse no.4.

Preparation of test materials

In order to administer the crude extract at dose of and 200 mg/kg body weight of mice, 50 mg of the extract was measured and triturated unidirectional way by the addition of three drops of

Tween-80. After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made 2.5 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of diclofenac at the dose of 40-mg/kg-body weight, 12.5 mg of diclofenac was taken and a suspension of 2.5 ml was made.

Table 3: Name of the test materials

Test samples	Group	Purpose	Dose (mg/kg)	Route of administration
Ethanol extract	I		250	
Pet ether extract	II	Test sample (*Curcuma zedoaria*)		Oral
Diclofenac	IV	Positive control	40	Oral
1%Tween 80 in saline	V	Control	10 ml/kg of body weight	Oral
Acetic acid (0.7%)	--	Writhing inducer		Intra-peritoneal

Procedure

At zero hour test samples, control (1% Tween-80 solution in saline) and diclofenac were administered orally with a feeding needle. After 30 minutes acetic acid (0.7%) was administered intra-peritoneally to each of the animals of all the groups. Forty five minutes interval was allowed for the absorption of the administered samples. Five minutes after the administration of acetic acid, number of squirms or writhing were counted for each mouse for fifteen minutes.

Counting of writhing

Each mouse of all groups were observed individually for counting the number of writhing over 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly two half writhing were taken as one full writhing.

Table 4: Analgesic activity of fractions of *Curcuma zedoaria* on acetic acid induced writhing on mice

Animal group	Writhing count				Mean	% writhing	% inhibition
Control	23.00	20.00	22.00	19.00	21.00	100.00	0.00
Diclofenac	10.00	9.00	10.00	11.00	10.00	47.61	52.38
Sample I	3.00	6.00	4.00	15.00	7.00	33.33	66.67
Sample II	14.00	5.00	5.00	1.00	6.25	29.76	70.24
Sample III	3.00	12.00	2.00	4.00	5.25	25.00	75.00
Sample IV	4.00	6.00	5.00	3.00	4.50	21.42	78.57
Sample V	10.00	6.00	3.00	5.00	6.00	28.57	71.43

Sample I: Methanol with rhizome extract

Sample II: Pet ether with rhizome extract

Sample III: Methanol with leaves

Sample IV: Pet ether with leaves

Sample V: Stem extract

Antimicrobial activity of crude extract

Antimicrobial screening

The antimicrobial activity of the plant extracts were visualized by antimicrobial screening. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Antimicrobial activity was carried out by disc diffusion method.[24] Standard antibiotic (kanamycin) discs and blank discs were used as positive and negative control. The plates were kept at low temperature (4˚C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media. The plates were then inverted and incubated at 37˚C for 24 hours for optimum growth of the organisms. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter.

Test organisms

The microbial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive, gram-negative bacteria and fungi were taken for the test listed in the Table 5.

Table 5: List of test microorganisms

Gram positive Bacteria	Gram negative bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Aspergillus niger*
Bacillus subtilis	*Salmonella paratyphi*	*Sacharomyces cerevaceae*
Staphylococcus aureus	*Salmonella typhi*	
Sarcina lutea	*Shigella boydii*	
	Shigella dysenteriae	
	Vibrio mimicus	
	Vibrio parahemolyticus	

Table 6: Sample code of the extracts

Plant	Test Samples	Code
Curcuma zedoaria	Leaf extract with Methanol.	LM
	Leaf extract with Pet ether.	LPE
	Rhizome extract with Methanol.	RM
	Rhizome extract with Pet ether.	RPE

Culture medium

Nutrient agar medium was used to make subculture and to see antimicrobial activity to the test organisms.

Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The microbial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media. Three types of discs were used for antimicrobial screening.

Standard discs

In this investigation, Kanamycin (30µg/disc) standard disc was used as the reference.

Blank discs

These were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Preparation of Sample Discs with Test Samples

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petridish under the laminar hood. Then the discs were soaked with solutions of test samples and dried.

Standard Kanamycin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Diffusion and Incubation

The sample, standard antibiotic and control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test microorganisms. The plates were then kept in a refrigerator at 4°C for about 24 hours to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours.

Determination of the Zone of Inhibition

After incubation, the antimicrobial activity of the test materials was determined by measuring the diameter of the zones of inhibition in millimeter using vernier caliper.

Table 7: Dose of the test samples in discs

Plant	Sample code	Sample	Dose (µg/disc)	Amount for 16 disc (mg)
Curcuma zedoaria	Et	Ethanol extract of the fruit	400	6.4
	Cl	Chloroform extract of the fruit	400	6.4

Table 8: Antimicrobial activity of the extracts

S. No.	Name of microorganism	Diameter of Zone of Inhibition (mm)				RM (400 µg/disc)	RPE (400 µg/disc)
		Standard antibiotic (Kanamycin) (30 µg/disc)	LM (400 µg/disc)	LPE (400 µg/disc)	Stem extract (400 µg/disc)		
	Gram-positive						
1	*Bacillus sereus*	38	12	12	7	15	14
2	*Bacillus megaterium*	38	12	11	7	13	11
3	*Bacillus subtilis*	38	12	11	7	13	11
4	*Staphylococcus aureus*	37	12	11	7	13	11
5	*Sarcina lutea*	37	12	12	7	13	11
	Gram -negative						
1	*Salmonella paratyphi*	37	12	11	7	13	12
2	*Salmonella typhi*	37	12	11	7	13	11
3	*Vibrio parahemolyticus*	38	12	11	7	13	11
4	*Vibrio minicus*	38	12	11	7	13	11
5	*E. coli*	38	12	11	7	13	11
6	*Shigella dysenteriae*	38	12	11	7	13	11
7	*Pseudomonas aureus*	38	12	12	7	13	11
8	*Shigella boydii*	37	12	12	7	13	11
	Fungi						
1	*Saccharromyces cerevaceae*	38	12	12	7	13	12
2	*Candida albicans*	38	12	11	7	13	12
3	*Aspergillus niger*	38	11	10	7	14	14

LM = Leave extract with Methanol; LPE= Leave extract with Pet ether; RM = Rhizome extract with Methanol; RPE= Rhizome extract with Pet ether.

RESULT AND DISCUSSION

Findings of analgesic activity

The crude extracts of Methanol and Pet ether were subjected to the study of analgesic properties using acetic acid induced writhing effect method. The result of this experiment is given in table 4. The result showed that the Methanol and Pet ether extracts had mild analgesic property (having a writhing inhibition of 51.7% and 50% respectively), while carbon tetrachloride extract did not show significant analgesic property (having a writhing inhibition of 30 %)

Findings of antimicrobial activity

The crude extracts of Methanol and Pet ether extract were screened for antimicrobial property using disk diffusion method. Gram positive and gram negative bacteria and fungi were used here as test organism. The result obtained were compared with a standard antimicrobial drug – kanamycin. The result of antimicrobial screening has been shown in table 8. The Methanol extract also showed mild sensitivity to several gram positive, gram negative bacteria & Fungi (zone of inhibition 7-10 mm).and slightly to highly sensitive to fungi (zone of inhibition 8-40 mm). The pet ether extract Crystal (Found after adding CCl_4) showed mild sensitivity to only a gram negative bacteria (*Shigella boydii*) having zone of inhibition 7 mm.

CONCLUSION

The methanol and pet ether soluble fractions showed mild analgesic and antimicrobial activity. It may support the traditional use of this plant in various diseases. It can be predicted that, further investigation with this plant may result in some innovative findings.

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