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Phyto-pharmacological screenings of two Rubiaceae family plants

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In the present study, the ethanol extracts of two plant species namely *Hymenodictyon excelsum* Roxb. Wall and *Mussaenda corymbosa* Roxb. from the Rubiaceae family were screened for their phytochemical and pharmacological activities. Phytochemical investigations revealed the presence of important chemical moieties such as alkaloids, glycosides, tannins, falvonoids, saponins, reducing sugars etc. Extracts of *M. corymbosa* (EMC) showed potent 1,1-diphenyl-1-picrylhydrazyl (DPPH) inhibition at 100 µg/ml. In the antimicrobial activity test by disc diffusion method, the extract of *H. excelsum* (EHE) showed poor antibacterial activity but good antifungal activity and it strongly inhibited the species, *Trichophyton* sp. Whereas, EMC at dose of 500 µg/disc showed strong inhibition against *Bacillus subtilis, Bacillus megaterium, Salmonella typhi* but it was inactive against the test fungi. EHE and EMC showed potent anti-inflammatory, membranstabilizing, analgesic, antipyretic, and moderate amylase inhibition activities. *H. excelsum* showed stronger central nervous system (CNS) depressing activity on Swiss mice compared to EMC. In conclusion, the plant species may be considered as a medicinal plant and therefore its folk use and further exploitation.

Key words: Investigantions, *Hymenodictyon excelsum*, *Mussaenda corymbosa*, pharmacological, phytochemical.

INTRODUCTION

Plants are used as medicine since time immemorial. These can be used as taxonomic markers for discovery of new therapeutic compounds (Cox, 1990). The use of plants as traditional medicine is widely accepted and practiced by the Villagers, Vaidyas, Ojhas, and some other elderly people, and the knowledge of it is culturally forwarded to the next generation. It is anticipated that plants can provide potential bioactive compounds for the

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development of new 'leads' to combat various diseases. Considering the vast area of potentiality of plants as sources for drugs and taking into account the local traditional uses, a systematic investigation was undertaken to establish the major classes of phytochemical and pharmacological activity of *Hymenodictyon excelsum* Roxb. Wall (a deciduous tree), which is traditionally used as febrifuge, and *Mussaenda corymbosa* Roxb. (an erect shrub) with traditional use in malaria, but this has no written evidences. However, *H. excelsum* has been evident for its good number of important phytoconstituents (Stanley and Lionel, 1916; Stanley et al., 1918; Rao et al., 1988; Joshi and Baxi, 1990; Joshi and Baxi, 1993; Parichat et al., 2009). Methanolic bark extract of *H. excelsum* also showed antimicrobial evidence against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans* and the leaves for antiinflammatory activity (Khairunnisa and Karthik, 2014). In addition it is also evident for its (*H. excelsum*) cytotoxic activity (Raushanara et al., 2014; Khairunnisa and Karthik, 2014). Both plants belonging to the family of Rubiaceae.

MATERIALS AND METHODS

Plant collection and identification

For the investigation, the bark of *H. excelsum* and leaves of *M. corymbosa* was collected from National Botanical Garden of Bangladesh and was identified by the taxonomist from Bangladesh National Herbarium. Two voucher specimens were deposited there, containing accession number 39547 and 39548 for *H. excelsum* and *M. corymbosa,* respectively. Bark of *H. excelsum* and leaves of *M. corymbosa* were collected and dried (temperature not exceeding 50°C), then the dried samples were subjected to course grinding. The powdered material was extracted with ethanol (95%) by the Soxhlet apparatus (Quickfit, England) for 10 h. Then the extracts were filtered through a cotton plug followed by Whatman filter paper no. 1. The extracts were then concentrated by evaporating the solvent. The yield of the crude *H. excelsum* and *M. corymbosa* extracts were 13.4 and 12.4%, respectively.

Preliminary screening for phytoconstituents

Preliminary phytochemical screenings was carried out as described by Khandelwal (2003) and the results have been given in the Table 1.

Screening for antioxidant activity by DPPH scavenging method

Antioxidant activities of the crude extracts were determined by the slightly modified method described by Manzocco et al. (1998). For this, stock solutions of the plant extracts and standard ascorbic acid were prepared in ethanol, with a concentration range from 100 to 1.56 µg/ml. To the diluted sample (0.1 ml), 2.9 ml of 0.004% 1,1-diphenyl-2-picryl hydrazoyl (DPPH) ethanolic solution was added. Then the contents were mixed properly and allowed to stand in the dark for 20 min to complete the reaction. The absorbance was determined at 517 nm. Results were expressed by the following equation.

$$\% \text{ inhibition of DPPH radical} = [(A_{br} - A_{ar})/A_{br}] \times 100$$

Where, A_{br} is the absorbance of DPPH free radicals before reaction and A_{ar} is the absorbance of DPPH free radicals after reaction.

Screening for antimicrobial activity

The antibacterial and antifungal action of the crude extracts was screened by the disk diffusion method described by Bauer et al. (1966). The test was conducted against 4 Gram positive (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus* and *Staphylococcus aureus*) and 6 Gram negative (*Escherichia coli*, *Shigella dysentariae*, *Shigeela sonnei*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholera*) species of pathogenic bacteria and 7 pathogenic fungi (*Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton* sp., *Microsporum* sp., *Cryptococcus neoformans)*. Microorganisms were collected as pure subculture from Microbiology Lab., Department of Pharmacy, BGC Trust University, Chittagong, Bangladesh. Azithromycin and fluconazole were taken as standards for antibacterial and antifungal tests, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeters (mm).

Minimum inhibitory concentrations (MIC) determination

Serial tube dilution technique (Andrews, 2001) in broth medium (NBM) (Hi Media Laboratories Ltd., India) was carried out for the evaluation of the minimum inhibitory concentration of the crude extracts. A half-fold dilution from 1000 to 7.81 µg/ml was performed for the MIC test. In brief, after performing dilution with the samples, aseptically 10^5 colony forming units of each organism were transferred to the sterile NBM containing tubes. Then the volume was adjusted with sterile NBM up to one milliliter (1 ml). Tubes were then incubated in an incubator (for bacteria: 24 h at 37 ± 1°C; for fungi: 72 h at 25 ± 1°C).

Screening for *in-vitro* anti-inflammatory and membrane stabilizing activity

This test was conducted by the Hypo-saline induced hemolysis method described by Vadivu and Lakshmi (2008). In this occasion, fresh human RBC was reconstituted as 10% suspension human red blood cell reconstitution (HRBC) in isosaline (0.9% NaCl, pH 7.4). The assay mixture contains 1 ml phosphate buffer (pH 7.4, 0.15 M), 2 ml hypo-saline (0.36%), 0.5 ml HRBC suspension (10% v/v) with 0.5 ml of test sample. A range of concentrations were tested as mentioned in Table 3. Acetyl salicylic acid (ASA) and distilled water (DW) were taken as positive and negative controls (NC). After incubation at 37°C for 30 min, the reaction mixtures were centrifuged and supernatant was collected for spectrophotometric analysis at 560 nm. Activity was measured by the following equation.

Inhibition (%) = 100 - [(absorbance of test solution) ÷ (absorbance of control) × 100]

Screening for antinoceptive activity on Swiss albino mice

The analgesic activity of the crude extracts was determined by slightly modified method of Eddy and Leimbach (1953). Diclofenac-Na was used as standard. Young Swiss mice (either sex, 18 to 24 g) were divided into six groups of 5 animals in each. Group I served as NC (DW, i.p.). Group II served as standard and were injected diclofenac sodium (9 mg/kg) intraperitonially. Group III and IV were

Table 1. Phytoconstituents found in the ethanolic extracts.

Phytoconstituent		Alkaloids	Glycosides	Steroids	Tannins	Falvonoids	Saponins	Reducing sugars	Gums
Consequence	EHE	+++-	++	--	++-	+	+	++	+
	EMC	+++-	--	++	---	+	+	++	+

*(+) = Presence; (-) = Absence; number indicates number of test performed. EHE: Ethanolic extract of *H. excelsum*; EMC: Ethanolic extract of *M. Corymbosa*

treated intraperitonially with ethanol crude extract of *H. excelsum* (EHE) and *M. corymbosa* (EMC) at the doses of 500 and 250 mg/kg body weight, respectively. The animals were individually placed on the hot plate (55 ± 2°C), 15 min after respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first (latency). Moreover the number of times jumped and number of times the paw licked was noted as a pain stimulus for 2 min. The cut off time for the reaction was 15 s.

Screening for neuropharmacological activity

For this purpose, open field test (OFT) (Cícero et al., 2008), hole cross test (HCT) (Takagi et al., 1971), light dark test (LDT) (Rogoz et al., 2003) and swing test (Islam et al., 2014) were adopted for the evaluation of neuropharmacological activity of the crude extractives on Swiss mice (either sex, 18 to 24 g, n = 5). To do that, two doses (250 and 500 mg/kg, i.p.) of the ethanol crude extracts of EHE and EMC were taken. For vehicle distilled water (10 ml/kg, i.p) and for standard, diazepam (4 mg/kg, i.p.) was administered to the experimental animals. After the sample administration, animals were observed for 5 min in each test apparatus (Table 7).

Screening for anti-atherothrombotic activity

The thrombolytic activity of the extract was evaluated by the earlier described method of Prasad et al. (2006) using streptokinase (SK) 1500000 I.U and ethanol as standard and NC. For this study blood was collected from 10 healthy volunteers and distributed into pre-weighed (W_1) micro-centrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 min and then weight (W_2) was taken. The weight of clotted blood (ΔW) was taken by subtracting the pre-weight and the weight of clotted blood containing tube. Then 100 µl test samples were added to the clot containing tubes marked. Each crude sample was tested for five doses (500, 250, 125, 62.5 and 31.25 µg). Similarly, 100 µl of streptokinase (1,500,000 U/vial/10 ml) and 100 µl of ethanol were added to the Standard and NC tubes. Then all the tubes were incubated at 37°C for 90 min. After incubation, fluid released was removed carefully without disrupting the clot, and tubes were again weighed for getting the weight variation among the pre-weight and final weight (W_3) that was achieved for clot lyses (thrombolysis).

Screening for antipyretic activity

Antipyretic activity of the EHE/EMC was measured by slightly modified method described by Adams et al. (1968). Swiss mice (either sex, 18 to 24 g, n = 5) were fasted overnight before the experimentation and were grouped as Gr. I to IV for extracts, Gr. V for standard and Gr. VI for NC. Pyrexia was induced by brewer's yeast suspension (10 ml/kg, 30% (w/v), i.p.). Twenty four hours after injection, the rectal temperature of each mouse was measured by using a digital thermometer. Animals that showed an increase in temperature of at least 0.7°C were used for the experiment. After sample administration rectal temperature was measured at 1, 2 and 3 h. Paracetamol (150 mg/kg, i.p.) and DW (10 ml/kg, i.p.) were used as standard and NC, respectively.

Screening for anti-amylase activity

The α-amylase inhibiting activity was measured using the starch-iodine method by Komaki et al. (2003). Acarbose at the dose of 50 µg/ml was taken as standard. For standard, acarbose serial dilutions were carried out to obtain concentration range from 15.625 to 250 µg/ml, and for plant extract concentration range from 31.25 to 500 µg/ml in DW. The percentage inhibition was calculated by comparing to the vehicle (NC) marked group. The inhibition of amylase activity was measured by the following equation.

Percentage inhibition of enzyme activity = (A-C) × 100/ (B-C)

Where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of NC (no extract).

Statistical analysis

All results are presented as mean ± standard deviation (SD) values. The data were analyzed by means of analysis of variance (ANOVA) followed by *t*-Student–Newman–Keuls's as post-hoc test. Data were analyzed using the Graph Pad Prism software (version 6.5) and experimental groups were compared with the control group. The levels statistical significance ranged with $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS AND DISCUSSION

Plants from Rubiaceae family possess variety of chemically important functional moieties that have essential pharmacological activities. Most of the tribal medical healers in Bangladesh use both plant species (*H. excelsum* and *M. corymbosa*) for certain disease management. Thus the present study on EHE and EMC was conducted to determine pharmacological activities of both the plants and their comparative results. The EHE and EMC possess good antioxidant, antimicrobial, anti-inflammatory, analgesic, depressing, antipyretic and moderately antiamylase and atherothrombolytic activities.

From the data depicted on Table 1, preliminary screening for secondary metabolites of both the crude extracts revealed the presence of alkaloids, falvonoids, saponins, reducing sugars and gums in both the extracts as well as glycosides and tannins in *EHE* and sterioids in EMC. In the qualitative antioxidant test by DPPH

Table 2. Antioxidant activities by crude extract and standard.

Concentration (µg/ml)	Percent inhibition of DPPH activity by extracts/standard		
	EHE	EMC	AC
100	50.25	88.82	94.75
50	24.57	55.04	93.62
25	21.01	14.37	90.78
12.5	04.67	08.72	50.50
6.25	02.58	05.53	32.34
3.125	01.35	02.09	13.33
1.5612	00.37	01.60	12.20
IC_{50} (µg/ml)	36.91	41.72	9.302

Values are expressed as percentage of inhibition of DPPH activity; AC: Ascorbic acid.

scavenging method, both the crude samples showed yellow color region coated by purple color background immediately after spraying DPPH solution. Color was observed in a UV-chamber both short (254 nm) and long (360 nm) ultra-violet range. During the quantitative test, both the crude samples including standard, ascorbic acid produced a dose graded DPPH scavenging inhibition. Percentage inhibition 94.75, 88.82 and 50.25 were showed by the highest dose (100 µg/ml) of the ascorbic acid, EMC and EHE, respectively. From the tabular presentation (Table 2) it is clear that EMC is more potent than the EHE and it showed a similar inhibitory activity of standard, ascorbic acid. IC_{50} of the test samples, EMC and EHE and standard were 41.72, 36.91 and 9.302 µg/ml. The presence of phenolic compounds could be one of the main reasons for the antioxidant activity.

In the antibacterial sensitivity test, it was observed that the EHE and EMC produced significant zone of inhibition. The highest zone of inhibition produced by EHE was 10 mm against *Shigella dysenteriae* at 50 µg/µl but with no inhibition at 25 µg/µl, and then followed by 7 mm against *Pseudomonas aeruginosa* at 50 µg/µl but with no inhibition at 25 µg/µl. The extract did not produce inhibition to the other tested bacteria. Whereas in case of crude extract of EMC the highest zone of inhibition produced was 26 mm against *B. megaterium* at 50 µg/µl and 18 mm at 25 µg/µl. Then followed by 24 mm (50 µg/µl), 17 mm (25 µg/µl), against *B. subtilis*, 18 mm (50 µg/µl), 8 mm (25 µg/µl) against *Salmonella typhi* and 8 mm (50 µg/µl), 7 mm (25 µg/µl) against *Vibrio cholera,* respectively (Table 3).

In the antifungal sensitivity test, it was observed that the ethanolic crude extract of EHE produced significant zone of inhibition against some of the clinical fungal species but the EMC was inactive against all the other tested fungi. The EHE extract produced highest zone of inhibition (26.25 mm) at 50 µg/µl and 24.47 mm at 25 µg/µl against the *Trichophyton* sp. Then followed by 10.37 mm (50 µg/µl) and 12.53 mm (50 µg/µl) by *Pityrosporum ovale* and *Aspergillus niger* respectively. But the extract produced no inhibition to the other tested fungi (Table 3). EMC showed no sensitivity against the tested fungal species.

The EHE inhibited the growth of bacterial species *P. aeruginosa* and *Shigella dysenteriae* significantly at the dose of 125 µg/ml and that of fungal species *A. niger*, *Pityrosporum ovale, Trichophyton* sp. at 125, 250 and 125 µg/ml concentrations, respectively. But the extract was found to be inactive against other bacterial and fungal strains. In case of EMC the growth of bacterial species *B. subtilis*, *B. megaterium*, *S. typhi* and *V. cholera* were inhibited at concentrations of 125, 250, 250 and 125 µg/ml, respectively. But the extract was found to be inactive against other bacterial and fungal strains**.** The presence of flavonoid, tannins, saponins and steroids could also be one of the reasons for the antimicrobial activity (Table 3).

During the *in vitro* anti-inflammatory test and membrane stabilization test the crude ethanol extracts of EHE and EMC, standard (acetyl salicylic acid) produced percentage hemolysis and inhibited percentage hemolysis by an increasing order of their applied doses. EHE produced hemolysis percentage 13.24 by 500 µg/ml, and then followed by 20.34 and 6.62% at 500 and 250 µg/ml by EMC and ASA, respectively. IC_{50} of the standard, ASA, ethanolic crude extracts of *H. excelsum* and *M. corymbosa* were 19.79, 35.79 and 41.84 µg/ml, respectively. A dose response relationship was observed during the experiment. EHE produced percentage inhibition hemolysis of 86.76 by 500 µg/ml and then followed by 79.66 and 93.38% at 500 and 250 µg/ml by EMC and ASA, respectively (Table 4).

Data presented in the (Table 5) shows the analgesic effect produced by EHE is more potent than the EMC. EHE at the dose of 500 mg/kg showed 32.33 times jumping, 6.33 times paw licking, 11.97s latency period for jumping and 45.47s latency period for paw licking, whereas EMC at the same dose showed 35.33 times jumping, 7.33 times paw licking, 9.17s latency period for jumping and 14.77 s latency period for paw licking. It should also be mentioned that, EHE produced significant (P<0.05) analgesic activity compared to the standard,

Table 3. Antimicrobial activity of different extracts of *H. excelsum* and *M. corymbosa*.

Test microorganisms	Zone of Inhibition (mm)			MICs (µg/ml)	
	µg/disc	EHE	EMC	EHE	EMC
Bacillus subtilis	500	Ni	24	Nd	125
	250	Ni	17		
Bacillus megaterium	500	Ni	26	Nd	250
	250	Ni	18		
Pseudomonas aeruginosa	500	7	Ni	125	Nd
Salmonella typhi	500	Ni	8	Nd	250
	250	Ni	7		
Vibrio cholera	500	Ni	18	Nd	125
	250	Ni	8		
Pityrosporum ovale	500	13	Ni	250	Nd
Trichophyton sp	500	26	Ni	125	Nd
Microsporum sp	500	24	Ni	125	Nd

NI: No inhibition; Zones of inhibition less than 7 mm were considered as poor activity and were discarded; Nd: Not detected.

Table 4. Anti-inflammatory and membrane stabilizing activities of the controls and crude test extracts.

% hemolysis inhibition (test samples)			% hemolysis inhibition (standard)	
Concentration (µg/ml)	EHE	EMC	Concentration (µg/ml)	ASA
500	86.76	79.66	250	93.38
250	84.56	77.69	125	86.27
125	77.21	75.98	62.5	78.68
62.5	74.75	54.41	31.25	62.5
31.25	34.31	28.33	15.625	38.37
IC_{50} (µg/ml)	35.79	41.84		19.79

Values are expressed as percentage inhibition of hemolysis; ASA: Acetyl salicylic acid.

diclofenac-Na at a dose of 9 mg/kg. Moreover analgesic effects have already been established in flavonoids, tannins and alkaloids (Musa et al., 2008; Zulfiker et al., 2010) therefore it is possible that the anti-nociceptive effects observed in the extracts may be attributed due to its phytochemical constituents.

During the neuropharmacological study it was observed that both crude extracts at the dose of 500 mg/kg produced reduced number of square cross, hole cross, light and dark residence, and swing by OFT, HCT, LDRT and ST tests when compared to the standards, diazepam and control (DW) groups. From Table 6, it is evident that both the EHE and EMC showed more depressing activity at the dose of 500 mg/kg rather than 250 mg/kg. Moreover the EHE produced more depressing activity compared to EMC. The depressing activity of EHE was very much close to that of diazepam rather than EMC so it can be concluded that EHE comparatively possessed good depressing activity. The results were significant $P<0.05$ for the neuropharmacological study. Phytochemicals like saponins and flavonoids have been reported by several researchers (Won et al., 1980; Dubois et al., 1986; Amos et al., 2001; Musa et al., 2006) to be responsible for sedative and likewise to inhibit spontaneous motor activity in mice. Thus, it could be suggested that alkaloids, saponins and flavonoids detected in the plant extracts could be responsible for some of these neuropharmacological effects.

In the *in vitro* thrombolytic activity test the addition of 100 µl SK, as positive control to the clots showed 81.08%

Table 5. Effect of crude test extracts and controls on hot plate reaction in mice.

Treatment groups	Dose (mg/kg)	No. of times jumped	No. of times paw licked	Latency period (s)	
				Jumping	Licking
DW	10	47.00±16.3	6.00±2.83	31.33±11.8	19.67±8.1
Diclofenac-Na	9	37.67±2.83*	4.67±0.7*	4.33±2.1*	9.10±6.8*
EHE	500	32.33±17.7*	6.33±0.7*	11.97±6.04*	45.47±6.04*
	250	47.00±7.1*	8.00±7.1*	6.43±0.78*	17.95±14.63*
EMC	500	35.33±29.7*	7.33±1.41*	9.17±31.99*	14.77±12.36*
	250	55.33±19.8*	15.67±24.04*	4.83±0.81*	10.34±4.53*

*P<0.05; Values are expressed as mean ± SD (n=5).

Table 6. Neuropharmacological activity by open-field, hole-cross, light-dark tests and swing tests.

Treatment groups with doses		Number of field cross	Number of hole cross	Number of light-dark residence	Number of swing
Vehicle (DW 10 ml/kg)		160.00±0.17	30.67±9.29	31.2±9.29	21.33±2.12
Diazepam (4 mg/kg)		43.33±6.24[a]	15±2.65[c]	24 ±11.1[c]	14.00±1.53[c]
EHE	(500 mg/kg)	42.33±30.2[c]	14.00±6.1[c]	23±6.1[c]	6.33±2.5[b]
	(250 mg/kg)	113.33±20.8[c]	16.33±4.9[c]	27±3.8[c]	15.00±4.6[b]
EMC	(500 mg/kg)	92.00±10.50[c]	19.67±4.5[c]	30±2.3[c]	13.67±3.8[c]
	(250 mg/kg)	157.00±12.2[c]	28.67±2.1[c]	36±7.9[c]	20.67±4.2[c]

[a]*P<0.01*, [b]*P*<0.02, [c]*P*<0.05; Values are expressed as mean ± SD (n=5)

clot lysis. On the other hand clots when treated with 100 µl ethanol (NC) showed only negligible clot lysis (2.47%). After treatment of clots with 100 µl of crude ethanolic extracts of EHE the clot lysis obtained were 42.23, 38.62 and 36.54% at dose of 0.5, 0.25 and 0.125 mg/ml, respectively. Whereas, after the treatment of clots with crude ethanolic extracts of EMC clot lysis of 47.16, 40.17 and 39.99% were obtained by consecutive doses 0.5, 0.25 and 0.125 mg/ml, respectively. Moderate clotlysis activity was observed for both crude extracts when compared with control groups. The mean of percentage of clot lysis obtained by EMC is more than that of EHE, but in turn both are more than that of ethanol alone. In the present study the results are significant *P*<0.001 (Table 7).

The antipyretic activity test data presented in Table 8 shows that EHE produced more hypothermic activity than the EMC . However, EHE at the dose of 500 mg/kg reduced temperature (°F) by 1.3, 2.29 and 2.8; while the same dose of EMC reduced by 1.12, 1.71, and 2.07 at 1st, 2nd and 3rd h, respectively. It should be mentioned that, EHE produced significant (*P*<0.05) anti-pyretic activity compared to the standard, paracetamol at a dose of 150 mg/kg. Flavonoids are known to target prostaglandins which are involved in the pyrexia (Rajnarayana et al., 2001). The presence of flavonoids in the crude extracts may be contributory to its antipyretic activity. Moreover the antipyretic action of the ethanolic extracts may also be, due to the inhibition of prostaglandin synthesis, leading to the suppression of elevated plasma level, especially since the extracts had been shown to possess anti-inflammatory activities (inhibition of Prostaglandin E) (Sidaye et al., 2011). During the alpha amylase activity test, Table 9 showed that the consecutive doses of crude ethanolic extracts (31.25 to 500 µg/ml) and standard acarbose (15.625 to250 µg/ml) produced a dose graded inhibition of amylase activity. EMC showed better inhibitory activity compared to EHE and both the extracts showed moderate inhibitory activity in comparison to the standard acarbose. IC_{50} of acarbose, EHE, and EMC were 285.3, 87.87 and 5.76 µg/ml, respectively. Moreover significant results (*P*<0.001) were obtained from both the crude extracts when compared to positive control (acarbose). Recent studies have shown that phenolic phytochemicals exert anti-diabetic activity through inhibition of

Table 7. Anti-atherothrombosis activities by the crude extracts and controls.

Dose/tube	Percentage of clotlysis (test samples)		Percentage of clotlysis (controls)	
	EHE	EMC	SK (100 µl)	Ethanol (100 µl)
500 mg/100 µl	42.23±4.2*	47.16±8.8*		
250 mg/100 µl	38.62±8.1*	40.17±8.6*		
125 mg/100 µl	36.54±6.2*	39.99±12.4*	81.08±0.027*	2.47±8.33
62.5 mg/100 µl	18.11±4.4	21.13±8.8		
31.25 mg /100 µl	11.21±7.8	9.34±6.3		

*P<0.001 Values are expressed as mean±SD (n=10); SK: Streptokinase.

Table 8. Antipyretic activity on Brewer's yeast-induced pyrexia in Swiss mice.

Control/extracts	Dose	Temperature (°F) reduction		
		60 min	120 min	180 min
Control(DW)	10 (ml/kg)	0.57±0.15	0.27±0.10	0.13±0.03
Paracetamol	150 (mg/kg)	1.33±0.16^{c}	2.10±0.10^{a}	2.57±0.30^{b}
EHE	500 (mg/kg)	1.30±0.26^{c}	2.29±0.14^{a}	2.80±0.24^{a}
	250 (mg/kg)	0.83±0.10^{c}	1.35±0.22^{c}	1.72±0.16^{a}
EMC	500 (mg/kg)	1.12±0.16^{c}	1.71±0.20^{b}	2.07±0.28^{a}
	250 (mg/kg)	0.65±0.27^{c}	1.00±0.11^{b}	1.35±0.26^{c}

^{a}P<0.01, ^{b}P<0.02, ^{c}P<0.05; Values are expressed as mean±SD (n=5).

Table 9. Anti-amylase activity by standard and extract.

% inhibition (test samples)			% inhibition (standard)	
Concentration (µg/ml)	EHE	EMC	Concentration (µg/ml)	Acarbose
500	62.29±0.02*	72.52±0.05*	250	90.49±0.03
250	19.37±0.01*	64.61±0.08*	125	87.12±0.02
125	14.29±0.01*	45.71±0.02*	62.5	85.14±0.03
62.5	11.90±0.03*	21.28±0.01*	31.25	74.77±0.04
31.25	7.35±0.02*	17.57±0.03*	15.625	68.06±0.02
IC_{50} (µg/ml)	285.3±0.03	87.87±0.05		5.76±0.10

*P<0.001; values are expressed as percentage inhibition of amylase activity.

carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase. Natural alpha-amylase inhibitors offer an attractive approach to the management of postprandial hyperglycemia by decreasing glucose release from starch (Kim et al., 2005). Several findings (Kwon et al., 2006; Apostolidis et al., 2007) suggest that phenolic synergies may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type 2 diabetes. Since the ethanolic crude extracts showed positive results for phenolic compounds (falvonoids and tannins) the amylase inhibition could be due to these constituents.

Conclusion

Ethnobotanical approach is one of the most common methods that are employed in choosing the plants for pharmacological study. The EHE and EMC suggested having some important phytochemicals and the presence of this phytochemicals makes this plant a potential candidate for use in different therapeutic conditions. In the present study ethanolic extracts of the two Rubiaceae family namely – the EHE. and EMC produced moderate antimicrobial activity. Moreover, both the crude extracts produced good antioxidant, anti-inflammatory, membrane

stabilization, analgesic, antipyretic and central nervous system (CNS) depressing activities. Also moderate anti-atherothrombotic and mild anti-amylase activities were observed during the pharmacological study. Though *H. excelsum* is evident for its antimicrobial activity but the *M. corymbosa* has been investigated for the first time. Further investigation is necessary prior to isolate and characterize the active principles.

Conflict of Interest

The authors have not declared any conflict of interest.

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