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Phytochemical and Neuropharmacological Activities of Different Fractional Extracts of *Firmianacolorata* R. Leaves

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Abstract

The subject of phytochemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry is closely related to both. Phytochemical and pharmacological screening of compounds of natural or synthetic has been the source of innumerable therapeutic agents. Plants are used in different countries as potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different pats used include seeds, root, stem, flower, fruit and leaves and modified plant organs.A systemic investigation was undertaken to screen the phytochemical and pharmacological activity of Firmianacolorata belonging to the family: Malvaceae.The leaves of F.colorata(Family: Malvaceae) were collected from Chittagong University. In the present study, ethanol, n-hexane and chloroform extractives ofFirmianacoloratawere screened for pharmacological and biological tests. It is observed that the ethanolic, chloroform and n-hexane extracts of F.coloratacontains glycosides, steroiods, alkaloids, saponins, reducing sugar, gums and amides.In this research work,the open field test after 120 minutes, square crossed by the control (42) and positive control group (16). Moreover, at the 500 mg/kg dose, Ethanol (30) and Chloroform (29) extract significantly decreases the movement then n-Hexane extract (34) shown in table 1. In the hole cross test after 120 minutes, the number of holes crossed by the control group (11) and positive control group (8). In this research work,the test samples of F.colorata at the dose of 400 mg/kg ethanol extract crossing movements of the experimental animals were almost similar to the control group (11). In this research work,chloroform (12) and n-hexane (13) extracts shows no activity in this method.

Keywords: Phytochemistry; Neuropharmacological Activities; Firmianacolorata; Chloroform.

Introduction

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants” (1). Although there are no apparent morphological characteristics in the medicinal plants that make them distinct from other plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plant which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycoside, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties. Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way (2): “A medicinal plant which, in one or of its organs, contains substances that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs”. Unfortunately, this definition of the WHO group includes only the medicinal plants whose therapeutic properties and chemical constituents have been established scientifically. But it does not take into consideration the vast majority of the medicinal plants, has been used in Traditional medicine for hundreds of years with reputation as efficacious remedies although there may not be scientific data to substantiate their efficacy (3). Nature appears to be a therapeutic cornucopia to treat the superfluity of diseases ranging from common cold multifarious type of illness since the dawn of civilization. Product of natural origins is often called “natural products”.Natural product include: an entire organism that has not undergone any kind of processing or treatment other than a simple process of preservation, part of an organism, an extract of an organism or part of an organism and exudates and pure compounds isolated from plants, animals or microorganisms. However, in most cases the term natural products refer to secondary metabolites, small molecules (4) produced by an organism that are not strictly necessary for the survival of the organism (5). Natural products have played a key role in drug discovery research, as many medicines are either natural product or derivatives. Indeed, it is estimated that

about 40% of all medicines is either natural products or their semi-synthetic derivatives (6). This may not be surprising as herbal medicine is a tradition of healthcare since ancient times and natural extracts screening has been one of the roots of drug discovery research, where erythromycin and rifampicin are a few well-known natural products based medicines. For bacterial infections, over 80% of all medicines in clinical use is either natural products or their derivatives, while for anti-cancer agents over 60% of all drugs is either natural products or derivatives thereof, examples of several potential lead molecules are vincristine, vinblastin, taxol, camptothecin, podophyllotoxin, combretastatins eta which have been isolated from plants for successful use in cancer treatment (7). The future of plants as source of medicinal agents for use in investigation, prevention and treatmentof diseases is very promising.Plants and man are inseparable. Plants not only provide us with food, shelter and medicine, but also life sustaining oxygen gas. In the present modern age of computers mankind has become almost entirely mechanical. Life has become tense in every occupation (8). A systemic investigation was undertaken to screen the phytochemical and pharmacological activity of Firmiana colorata belonging to the family: Malvaceae. It is used as traditionally by the people for the medical treatment of disease (9).

Materials and Methods

Table 1.

Different Test for Chemical Groups Present in The Plant Extracts.

Table 2.

From this research work,it is observed that the ethanolic, chloroform and n-hexane extracts of F.coloratacontains glycosides, steroiods, alkaloids, saponins, reducing sugar, gums and amides.

Swiss-albino mice of either sex, average weight 35 gm each were used for the experiment. The animals were divided into 4

Table 1. List of reagents and their chemical composition, used for the different chemical group test.

Reagents	Composition
Fehling's solution A	34.64g copper sulphate in 500ml distilled water
Fehling's solution B	173g potassium sodium tartrate and 50g sodium hydroxide in 500ml distilled water
Benedict's reagent	1000ml solution contains 173g sodium citrate, 100g of sodium carbonate and 17.3g blue vitriol
Salkowski reagent	Chloroform and few drops of conc. sulfuric acid
Libermann-burchared reagent	Chloroform, few drops of conc. sulfuric acid and 2-3 drops of acetic anhydride
5% Ferric chloride solution	5g ferric chloride in 100ml distilled water
10% Potassium dichromate solution	10g potassium dichromate in 100ml distilled water
Keller-Kiliani reagent	0.5ml glacial acetic acid, few drops of ferric chloride and conc. sulfuric acid
Mayer's reagent	100ml solution contains 1.358g mercuric chloride and 5g potassium iodide.
Dragendorff's reagent	100ml solution contains 8g bismuth nitrate, 20ml conc. nitric acid and 27.2g potassium iodide
Wagner's reagent	2g iodine and 6g potassium iodide in 100ml distilled water
Hager's reagent	1g picric acid in 100ml distilled water
Molisch's reagent	15 g alpha-naphthol in 100 ml alcohol or chloroform

Table 2. Tests for screening chemical groups in plant extracts.

Test name	Experimentation	Observation
Mayer's test	0.5 g extract was stirred with 5 ml 1% HO on a steam bath and then mixture was filtered, 1 ml of filtrate was treated with a few drop of Mayer's reagent.	White or creamy white precipitate indicates the presence of alkaloids.
Dragendorff's test	Small amount of sample on a watch glass + few drops of conc. + HCL + stir + 1 drop of Dragendorff's reagent.	Brick red precipitation indicates the presence of alkaloids.
Wagner's test	0.5 g extract was stirred with 5 ml 1% HCL on a steam bath and filtered.1 ml of filtrate was treated with a few drop of Wagner's reagent.	Brown or deep brown precipitate indicates the presence of alkaloids.
Hager's test	0.5 g extract was stirred with 5 ml 1% HCL on a steam bath and filtered. 1 ml of filtrate was treated with a few drop of Hager's reagent.	Yellow crystalline precipitate indicates presence of alkaloids.

Tannic acid test	1 ml solution of sample in a test tube + 0.5 ml of Wagner's reagent.	Characteristics precipitation was observed which was soluble in dil. HCL or ammonia solution; conforms the presence of alkaloids.
Test for Glycosides		
Test name	**Experimentation**	**Observation**
Salkowski's test	Few mg of sample in chloroform + 1-2 drops of conc. sulfuric acid.	Orange-reddish color at the junction of 2 layers; conforms the presence of glycosides.
Libermann-burchard's test	Few mg of sample in chloroform +1-2 drops of conc. sulfuric acid + 2-3 drops of acetic anhydride.	Violet color at the junction of 2 layers; conforms the presence of glycosides.
Test for Glycosides		
Test name	**Experimentation**	**Observation**
Salkowski's test	Few mg of sample in chloroform + 1-2drops of conc. sulfuric acid	Red color at the junction of 2layers; conforms the presence of steroids.
Libermann-burchard's test	Few mg of sample in chloroform + 1-2drops of conc. sulfuric acid +2-3 drops of acetic anhydride.	Light green color at the junction of 2 layers; conforms the presence of sterois.
Test for Tanins		
Tests name	**Experimentation**	**Observation**
Ferric chloride test	Dissolved about 0.5g of an alcoholic or aqueous extract of the plant material in 5-10ml of DW and filtered added few drops of 5% ferric chloride solution.	A blue, blue black, Green or blue green color or precipitation is produced in the presence of tannins.
Potassium dichromate test	5ml sample solution + 1ml 10% potassium dichromate solution	Orange precipitation indicates the presence of tannins
Test for Flavonoids		
Test name	**Experimentation**	**Observation**
Conc. HCL and alcoholic test	Small amount of alcoholic sample solution + 2-3 drops of conc. HCL	Immediate developments of red color indicates the presence of flavonoids
Test for Saponins		
Test name	**Experimentation**	**Observation**
Shake test (aq. solution)	1 ml aq. sample solution + 19ml distilled water + 15 minutes shake.	1 cm foam on liquid layer; conforms the presence of saponins.
Test for Reducing Sugar		
Test name	**Experimentation**	**Observation**
Fehling's test	2 ml aq. extract + 1 ml equal mixture of Fehling A and B solution + boil in a water bath.	Red or brick red precipitate conforms the presence of reducing sugar.
Benedict's test	0.5 ml aq. extract + 5 ml Benedict's reagent + boil in a water bath (5 min)	Red color precipitate conforms of reducing sugar.
Test for Gums		
Test name	**Experimentation**	**Observation**
Molisch's test	5 ml sample solution + Molisch reagent + sulfuric acid.	Red violet ring at the junction of 2 liquid layers; conforms the presence of gums.
Test for Amides		
Test name	**Experimentation**	**Observation**
Sodium hydroxide test	Few mg of the extract was taken and then few ml of 20% NaOH was added. The mixture was boiled for 15min	Liberation of NH3 gas which turns the red litmus to blue, indicates the presence of amides

groups of 3 animals each.

Preparation of Test Samples for Test, Standard and Control Groups

- For control, double distilled water was taken.
- For standard, Diazepam was dissolved in control (DDW) at the dose rate of 5mg/kg body weight.
- For test group, ethanol, chloroform and n-hexane extracts of F.colorata at the dose were dissolved in control (DDW) at the dose rate of 400 mg/kg body weight.

Open Field Test

The Open Field Test (10) was adopted for this test. The test was performed after slight modification of the previously proposed method. The aim of this study was to characterize the emotional behavior of mice using the square-cross test. The Swiss mice were grouped into four distinct groups with 3 mice in each namely - for extract dose (400 mg/kg), for standard (5 mg/kg) of diazepam was taken. Samples were administered orally to the mice. A 30 minutes interval was taken after drug administration then the numbers of square crossed by the mice were counted for 5 minutes. Then the samples data were compared with normal group for possible action of the extract.

Hole Cross Test

The Hole Cross Test (11) was adopted for this test. The test was performed after slight modification of the previously proposed method. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The Swiss mice were grouped into four distinct groups with 3 mice in each namely - for extract, dose (400 mg/kg), vehicle (DDW) and normal groups. Samples were administered orally to the mice. A 30 minutes interval was taken after drug administration then the numbers of hole crossed by the mice were counted for 5 minutes. Then the samples data were compared with normal group for possible action of the extract.

Results and Discussion

Screening for CNS Depressant Activity

Depression and anxiety disorders are the most common mental illness in human. It is not only life threatening but also negatively impacts on functional recovery from other neuropsychiatric disorders (4). Most of the drugs used nowadays have adverse side effects so the need for newer, better tolerated and more efficacious treatments is remaining high.The finding of the CNS depressants activities of the crude fractions of F. colorata are given below (Table 3).

Here, during the open field test after 120 minutes, square crossed by the control (42) and positive control group (16). But at the 500 mg/kg dose, Ethanol (30) and Chloroform (29) extract significantly ($p< 0.001$) decreases the movement then n-Hexane extract (34) shown in table 1.

The results obtained from analgesic activity test were expressed as the mean ± SEM. The results were analyzed using one-way ANOVA followed by using Dunnett's t-test. The statistical analysis was carried out with SPSS software. A difference was considered significant at $p<0.001$ (Table 4).

Conclusion

Traditional herbal medicines are becoming increasingly popular in worldwide. The efficacy of medicinal plants in disease management is established and the World Health Organization has recognized their use in the primary health care delivery system. In view of the complexity of herbal medicines and their inherent biological variations, it is necessary to determine their neuropharmacological activities. In this research work, the open field test after 120 minutes, square crossed by the control (42) and positive control group (16). But at the 500 mg/kg dose, Ethanol (30) and Chloroform (29) extract significantly decreases the movement then n-Hexane extract (34) shown in table 1. Moreover, in the hole cross test after 120 minutes, the number of holes crossed by the control group (11) and positive control

Table 3. CNS Depressant Activity by Open Field Test.

Control/Extract	Dose (mg/kg)	Number of Field Cross ± Standard Error of Mean				
		0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
Control (**Double distilled water**)	10 ml/kg	41 ± 2.53	42 ± 1.46	43 ± 2.66	44 ± 1.16	42 ± 1.54
Positive Control (**Diazepam**)	5 mg/kg	37 ± 0.89	35 ± 2.61	26 ± 0.89	23 ± 1.46	16 ± 1.74
Ethanol Extract of *F.colorata*	400 mg/kg	44 ± 1.46	38 ± 1.16	38 ± 0.89	31 ± 1.16	30 ± 2.32
Chloroform Extract of *F.colorata*	400 mg/kg	42 ± 1.16	39 ± 0.89	34 ± 0.89	35 ± 1.77	29 ± 0.89
n-Hexane Extract of *F.colorata*	400 mg/kg	46 ± 1.16	40 ± 1.16	44 ± 1.16	42 ± 1.54	34 ± 1.16

Table 4. CNS Depressant Activity of F. colorata Extracts in Multiple Comparisons by IBM SPSS Statistics Software.

Post Hoc Tests
Multiple Comparisons
Dependent Variable: CNS Depressant Activity Dunnett t (<control)

(I) Groups	(J) Groups	Mean Difference (I-J)	Standard Error	Significance	99.9% Confidence Interval
					Upper Bound
Diazepam	Control	-26.00001*	2.055	0	-15.855
EEFC	Control	-12.66668*	2.055	0	-2.522
NEFC	Control	-8.000	2.055	0.006	2.145
CEFC	Control	-12.33334*	2.055	0	-2.188

From the above table, the mean difference is significant (P) at the 0.001 level. Dunnett t-tests treat one group as a control, and compare all other groups against it. EEFC: Ethanol extract of F. colorata, NEFC: n-Hexane extract of *F. colorata*, CEFC: Chloroform extract of *F. colorata*.

Table 5. CNS Depressant Activity by Hole Cross Test.

Control/Extract	Dose (mg/kg)	Number of Hole Cross ± Standard Error of Mean				
		0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
Control (Double distilled water)	10 ml/kg	17 ± 0.89	15 ± 0.67	13 ± 1.86	14 ± 2.34	11 ± 1.21
Positive Control (Diazepam)	5 mg/kg	18 ± 1.54	15 ± 0.89	14 ± 1.16	10 ± 0.89	8 ± 0.89
Positive Control (Daizepam)	400 mg/kg	21 ± 0.89	18 ± 1.16	17 ± 0.89	15 ± 1.21	11 ± 0.34
Ethanol Extract of F.colorata	400 mg/kg	20 ± 0.89	19 ± 0.89	16 ± 1.46	15 ± 0.89	12 ± 0.89
Chloroform Extract of F.colorata	400 mg/kg	22 ± 0.89	20 ± 0.89	17 ± 1.21	15 ± 0.67	13 ± 0.89

From above table, In the hole cross test after 120 minutes, the number of holes crossed by the control group (11) and positive control group (8). But the test samples of F.colorata at the dose of 400 mg/kg ethanol extract crossing movements of the experimental animals were almost similar to the control group (11). As well as chloroform (12 and n-hexane (13) extracts shows no activity in this method.

group (8). In this research work,the test samples of F.colorata at the dose of 400 mg/kg ethanol extract crossing movements of the experimental animals were almost similar to the control group (11). And also chloroform (12) and n-hexane (13) extracts shows no activity in this method.

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