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Antioxidant, Analgesic, Antimicrobial, and Anthelmintic Activity of the Dried Seeds of *Bixa orellana* (L)

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ABSTRACT

The present study was performed to evaluate phytochemical groups, antioxidant, antibacterial, analgesic and anthelmintic activity of ethanol extract of seeds of *Bixa orellana* (Bixaceae). Preliminary phytochemical screening of ethanol extract exhibited the presence of reducing sugar, carbohydrate, saponin, terpenoids, tannins, alkaloids, acidic compound, proteins, steroids, and flavonoids. In the TLC-based qualitative antioxidant assay using DPPH, *B. orellana* extract showed the free radical scavenging properties that was indicated by the presence of yellow spot on a purple background on the TLC plate. In the quantitative free radical scavenging assay by DPPH, IC_{50} value was approximately 47 µg/mL, where IC_{50} value of the ascorbic acid was approximately 16 µg/mL. *B. orellana* extract displayed hydroxyl radical scavenging activity (SC_{50} = 157 mg/L) which was comparable to that of standard ascorbic acid (SC_{50} = 81 mg/L). The Total Phenolic Content (TPC) and Total Tannin Content (TTC) was found to be ~ 52 and ~ 25 mg GAE/gm of dried extract respectively using Gallic acid calibration curve. Total Flavonoid Content (TFC) was ~ 41 mg QE/gm of dried extract using Quercetin calibration curve. The extract showed antibacterial activity against *S. aureus, S. dysenteriae*, *P. aeruginosa*, *S. typhi*, and *E. coli* at the doses of 250 and 500 µg/disc in comparison with standard drug Fucloxacillin (30 µg/disc) and Ciprofloxacin (30 µg/disc). The extract showed significant ($p<0.01$) acute peripheral analgesic activity at the doses of 250 (40.34% writhing inhibition) and 500 mg/kg body weight (67.05% writhing inhibition) determined by acetic acid induced writhing method in mice as compared to Diclofenac sodium (79.55%). In anthelmintic activity test, time taken for paralysis and death at the concentration of 25 and 50 mg/mL was approximately 33 minutes and 39 minutes and 23 minutes & 30 minutes respectively whereas standard showed approximately 7 and 16 minutes at the dose of 15 mg/mL respectively against the parasite. The phytochemical groups present in this plant may be responsible for the aforementioned pharmacological effects.

Keywords: *Bixa orellana*, Phytochemical study, Antioxidant activity, Antibacterial activity, Analgesic activity, Anthelmintic activity.

INTRODUTION

Modern synthetic drugs are very effective in curing disease but cause a number of side effects. Therefore, researchers have motivated to develop new drugs from medicinal plants in recent years, especially due to the constant emergence of resistant to conventional drugs. *Bixa orellana* L. (Family: Bixaceae), commonly known as Annatta, Latkan, Belatihaldi, Utkana, Lip stick tree, Achiolte, has been investigated because of their numerous traditional uses for the treatment of many ailments.

B. orellana is native to tropical American area and cultivated in warm regions of the world. In Bangladesh it is cultivated in Chittagong Hill Tracts, sporadically in other districts [1]. It is a

tall shrub to small evergreen tree and 6–10 m (20–33 ft) high, trunk is up to 10 cm in diameter. Moreover it has evergreen ovate leaves with reddish veins; pink, white, or some combination flowers; two-valved, woody capsule covered either with dense soft bristles or a smooth surface fruit approximately 4 cm wide, appear in a variety of colors [2].

Traditionally the root bark of the plant is antiperiodic and antipyretic and also used in intermittent, remittent and continued fevers. Root is used in jaundice, and fever. Seeds are cordial, astringent, febrifuge, diuretic, laxative, digestive; anti-dysenteric, and also used in epilepsy, skin diseases and gonorrhoea. Fresh pulp (the colouring matter, surrounding the seeds) is astringent; applied to burns to prevent blisters and scars [3-5]. American Indians use the pulp to paint their body, which they believe, prevents mosquito bites. Seeds fatty oil is used in leprosy. Leaves are febrifuge, useful in jaundice, dysentery; decoction of the leaves is used as a gargle for sore throat. In developing countries the plant is also used as folk medicine for the treatment of common infections and as an anti-parasitic agent [6]. The seeds of this plant produce dyes which are most frequently used worldwide, not only in food products but also in the textile, paint, and cosmetic industries. So it is also known as "Lip stick tree" [7].

MATERIALS & METHODS

Collection and extraction

The seeds of *Bixa orellana* (L.) were collected from Komolgonj, Moulobibazar, Sylhet, Bangladesh in May 25, 2016 at the daytime. The sample was identified by the experts of Bangladesh National Herbarium, Mirpur, and Dhaka **(Accession No.: 43217 DACB)**. The collected seeds were shade-dried for about 15 days. The dried seeds were then powdered with the help of a suitable grinder. The seeds were extracted by cold extraction method. About 350 gm powder was soaked in 1500 mL ethanol in a glass container for fifteen days accompanying regular shaking and stirring.

Then the extract was separated from the plant debris by filtration with a piece of clean, white cotton cloth. The filtrate obtained was evaporated using rotary evaporator. After a few days, the concentrated extract (approx. yield value 1.29%) was transferred into a small beaker and the opening of the beaker was wrapped by a sheet of aluminum foil on which perforation was done. The extract thus obtained was stored in a cool, dark and dry place.

Animals

Young *Swiss albino* mice aged 4-5 weeks, average weight (28-35) gm were used for analgesic experiment. The mice were collected from the animal house of Jahangirnagar University, Savar, Dhaka-1342. They were kept in the animal house of the Pharmacy Discipline, Khulna University, Khulna under standard Laboratory condition (relative humidity (55-60)%, room temperature $(25 \pm 2)^0$C and 12 hours (light: dark cycle) for period of 14 days prior to performing the experiment. The animals were provided with standard laboratory food and tap water.

Microorganisms

For antibacterial test both gram positive and gram negative bacterial strains were taken. These bacteria were collected from the Microbiology Lab. of Pharmacy Discipline, Khulna University, Khulna. The live parasites, Nematodes and Trematodes were collected from freshly slaughtered cattle at local abattoirs for anthelmintic test and stored in 0.9 % Phosphate buffered saline (PBS) of pH 7.4.

Drugs

The standard drugs Flucloxacillin, Ciprofloxacin disc, Diclofenac sodium, and Albendazole were collected from local pharmacy in Khulna.

Identification of phytochemical constituents

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups [8]. Then, the extract was used for pharmacological screening.

Estimation of antioxidant components

DPPH free radical scavenging assay

DPPH free radical produces a violet solution in ethanol and shows a characteristic spectrum with a maximum absorbance close to 517 nm. Based on electron transfer reaction, free radical which is stable at room temperature is reduced to a light yellow or colorless ethanol solution in the presence of an antioxidant molecule by electron transfer [9].

DPPH based qualitative analysis was determined on the basis of their scavenging activity of DPPH free radical by TLC technique using commercially prepared TLC plate [10]. The sample and the standard ascorbic acid were spotted on TLC plate. The chromatogram was developed by ascending technique using three types of solvent systems i.e. non-polar (*n*-hexane: Ethyl acetate = 2: 1), medium polar ($CHCl_3$: CH_3OH = 5: 1) and polar ($CHCl_3$: CH_3OH: H_2O = 40:10:1) solvent system. The solvent system was allowed to move up to a previously marked line.

After drying the plates, they were viewed under UV detector both in short wavelength (254 nm) and long (366 nm) wavelength. The plates were then sprayed with 0.02 % ethanol solution of DPPH by spray gun. Finally the plates were dried with a current of air suitably.

DPPH based quantitative analysis of seeds of *B. orellana* was performed by microplate technique [11]. At first plant extract and ascorbic acid as positive control was dissolved in DMSO to make the required concentrations by dilution method. Then 10 µL of different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/mL) of plant extract and ascorbic acid were taken in each well of the microplate and 190 µL DPPH in methanol was added to each well. Here, 190 µL of DPPH was applied in two well of the plate as blank. After mixing for 5 minutes, the plate was kept standing at room temperature in light for 30 minutes, and finally the absorbance was measured at 517 nm using micro plate reader.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extract was measured based on the method of Halliwell, Gutteridge et al., [12] with a slight modification according to Jiang, Bank et al., [13] In this test 0.5 mL 2-deoxy-D-ribose solution was mixed with 12.5 µL of different concentrations (6.25, 12.5, 25, 50, 100, 200, 400, and 800 mg/L) of sample or standard. Then 1 mL of 200 µM FeCl3, 1 mL of 1.04 mM EDTA, 0.5 mL of 1 mM H_2O_2 was added to prepare the reaction mixture. After incubation at 37^0C for 1 hour, 3.75 mL of 2.8% TCA and 3.75 mL of 1% TBA were added and kept at 100^0C for 20 minutes. Finally the absorbance was measured at 530 nm against blank for each concentration.

Polyphenolic compound determination

Determination of total phenolic content and total tannin content

Total phenolic and tannin content was determined by Folin-Ciocalteau Colometry method [14]. In such case, Gallic acid is usually used as reference standard and the result is usually expressed as gallic acid equivalence [15].

For the determination of total phenolic content, 0.5 mL of different concentrations (0.15, 0.1, 0.08, 0.06, 0.04, 0.02 mg/mL) of standard and extract solution were taken separately into different test tubes. Then 5 mL FC reagent (1/10) and 7% Na_2CO_3 were added to every test tube. Then they were kept for 30 minutes at 40ºC temperature. After 30 minutes the UV absorbance was measured at 765 nm against blank for each concentration.

In Total Tannin Content determination, 0.1 mL of different concentrations (0.5, 0.4, 0.3, 0.2, 0.1 mg/mL) of standard and extract sample solution was taken separately in different test tube. Then 7.5 mL of distilled water and 0.5 mL of FC reagent was added to the test tube. After that 1mL of 35% Na_2CO_3 was added to the test tube and the solution was diluted Q.S. to 10 mL with distilled water. Then all test tubes vortex for 15 second and kept at room temperature for 30 minutes. Absorbance of the solution was measured at 725 nm against blank for each concentration.

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride method [16]. In such case, quercetin is usually used as reference standard and the result is usually expressed as quercetin equivalence.

In this test, 1 mL of different concentrations (1, 0.75, 0.5, 0.25, and 0 mg/mL) of standard and sample extract solution were taken separately into different test tubes. Then 4 mL of distilled water was added and 0.3 mL of 5% w/v $NaNO_2$ was added to every test tube and kept for 5 minutes. Then 0.3 mL of 10% w/v $AlCl_3$ solution and 2 mL of 1M NaOH were also added to every test tube and added distilled water Q.S. to10 ml of the solution. They were kept for 15 minutes at room temperature and the absorbance was measured at 510 nm against blank for each concentration.

Antibacterial activity test

The evaluation of antibacterial potential of ethanol extract of seeds of *Bixa orellana* was carried out by Disc Diffusion method using both gram positive and gram negative bacterial strains [17,18]. In these test two types of standard antibiotic disc was used as positive control such as Flucloxacillin antibiotic disc and the commercial Ciprofloxacin antibiotic disc. For preparing the Flucloxacillin antibiotic disc, a 250 mg flucloxacillin capsule was used and 3 mg equivalent weight of the powder was then dissolved in 1 mL 50% ethanol to prepare standard solution [19]. Sample impregnated discs (250 and 500 µg/disc), standard antibiotic discs (30 µg/disc) and negative control discs (10 µL 50% ethanol/disc) were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then incubated at 37°C for (16-18) hours for observation. After proper incubation, the antibacterial activity of the test agent was determined by

measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

Analgesic activity test

The analgesic activity of the sample was investigated using acetic acid induced writhing method in mice [20]. Experimental animals were randomly selected and divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting of five (05) mice in each group.

Test samples, positive and negative control solution were given orally by using feeding needle. Control group received 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received Diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with test sample at the dose of 250 and 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances.

Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animal of a group. After an interval of 5 minutes, which was given for absorption of acetic acid, number of writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing.

Anthelmintic activity test

Anthelmintic activity of the plant extract was determined according to Hossain et al. [21]. For this test four petridishes were taken denoted as Control group, Positive control group and Test group I and Test group II consisting of six parasites in each petridish. 10 mL of 0.1 % Tween-80 in PBS as negative control, Albendazole at the dose of 150 mg/10 mL as positive control and suspension of the ethanol extract at the dose of 250 and 500 mg/10 mL were taken in different petridishes. Time taken for paralysis for each parasite was recorded when no movement was observed unless shaken vigorously. Time taken for death for each parasite was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli.

STATISTICAL ANALYSIS

Student's t-test was used to determine significant differences between the control group and test group.

RESULTS

In the preliminary phytochemical analysis of seeds of *B. orellana* extract exhibited the presence of reducing sugar, carbohydrate, saponin, terpenoids, tannins, alkaloids, acidic compound, proteins, steroids, and flavonoids (Table 1).

Table 1: Results of preliminary phytochemical analysis.

Plant Extract	Alkaloids	Glycosides	Steroids	Carbohydrate	Proteins	Tannins	Saponins	Flavonoids	Reducing sugar	Acidic compound
Ethanol extract of *Bixa orellana*	+	-	+	+	+	+	+	+	+	+

+ = Presence - = Absence

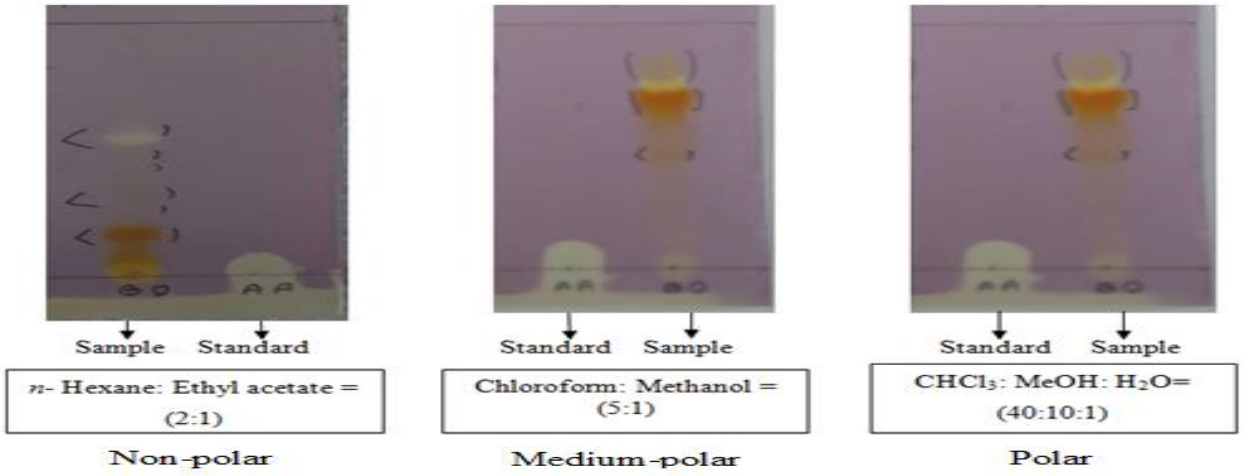

Figure 1: Comparison of TLC plate for *B. orellana* with the standard after applying DPPH.

Antioxidant activity test

In DPPH Free Radical Scavenging Assay the presence of strong yellow spot on a purple background of *B. orellana* extract in comparison with ascorbic acid (sample) on the TLC plate (Figure 1), an indication of free radical scavenger, helped us to quantify it through quantitative analysis using DPPH. In the quantitative analysis, *B. orellana* extract revealed free radical scavenging activity with approximate IC_{50} value of 47 µg/mL where IC_{50} of the standard ascorbic acid was approximately 16 µg/mL (Table 2 and Figure 2).

Table 2: %inhibition of standard and sample at different concentration.

% Inhibition	1 µg/mL	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL	64 µg/mL	128 µg/mL	256 µg/mL	512 µg/mL
Standard	5.67	13.1	21.38	33.37	51.92	68.36	81.71	90.48	95.18	96.54
Sample	6.18	11.87	18.67	24.47	30.28	37.45	45.98	54.01	64.28	75.77

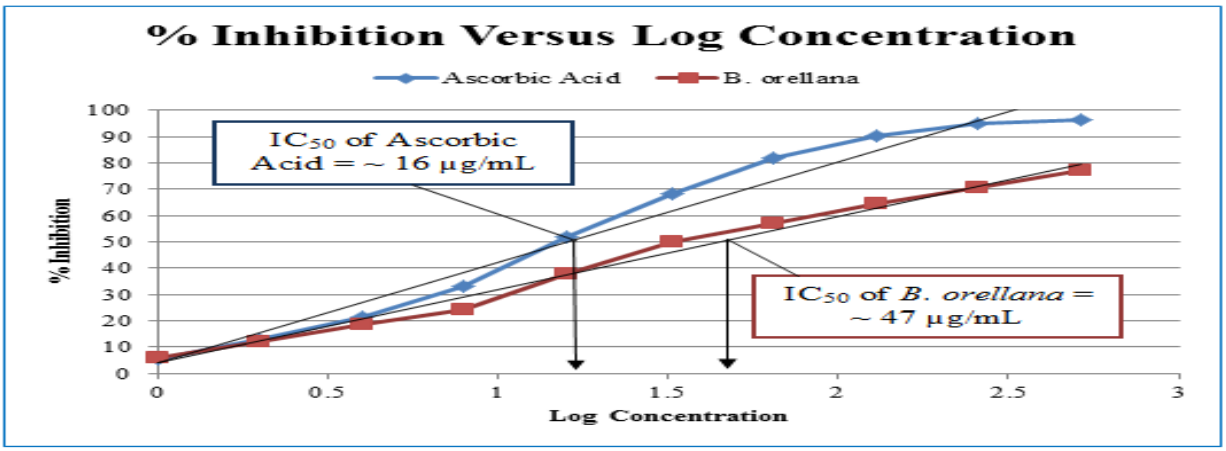

Figure 2: Comparison of % inhibition vs. log concentration graph for standard (ascorbic acid) vs. *B. orellana* in DPPH scavenging assay.

The *B. orellana* extract possessed moderate hydroxyl radical scavenging ability (SC_{50} = ~ 157 mg/L) that was comparable to ascorbic acid (SC_{50} = ~ 81 mg/L) (Table 3 and Figure 3).

Table 3: % scavenged of standard and sample at different concentration.

% Scavenged	6.25 mg/mL	12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	400 mg/mL	800 mg/mL
Standard	8.69	15.95	27.64	40.31	52.28	65.95	79.34	92.45
Sample	4.98	10.83	16.67	30.48	46.58	57.12	64.1	76.92

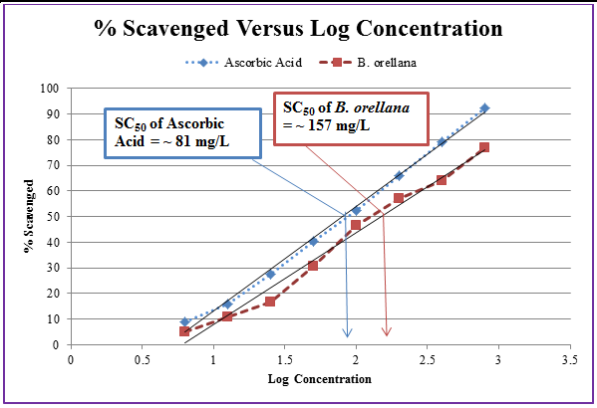

Figure 3: Comparison of % scavenged *versus* log conc. for ascorbic acid and *B. orellana.*

In polyphenolic compound determination, the Total Phenolic Content (TPC) & Total Tannin Content (TTC) was found to be ~ 52 mg GAE/gm (Table 4 and Figure 4) and ~ 25 mg GAE/gm (Table 4 and Figure 5) of dried extract respectively using Gallic acid calibration curve and Total Flavonoid Content (TFC) was ~ 41 mg QE/gm of dried extract using Quercetin calibration curve (Table 4 and Figure 6).

Antibacterial activity test

The antibacterial activity of *B. orellana* extract was performed using two different antibiotics as positive control. At the doses of 250 and 500 µg/disc, the extract showed antibacterial activity against the experimental bacterial strains namely *Shigella dysenteriae*, *Pseudomonas aeruginosa* (*Pseudomonas aeruginosa* didn't show activity at 250 µg/disc), *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* as compared with Flucloxacillin (30 µg/disc) and Ciprofloxacin (30 µg/disc). The maximum zone of inhibition was found against *Staphylococcus aureus* that was 20.5 and 26.5 mm when Flucloxacillin was used as positive control (Table 5 and Figure 7) and 21 and 27 mm when Ciprofloxacin was used as positive control (Table 6 and Figure 8) at the dose of 250 and 500 µg/disc respectively.

Table 4: Determination of phenolic content of *B. orellana* extract.

Sample	Total phenolic content (mg GAE/gm)	Total tannin content (mg GAE/gm)	Total flavonoid content (mg QE/gm)
***B. orellana* extract**	~ 52	~ 25	~ 41

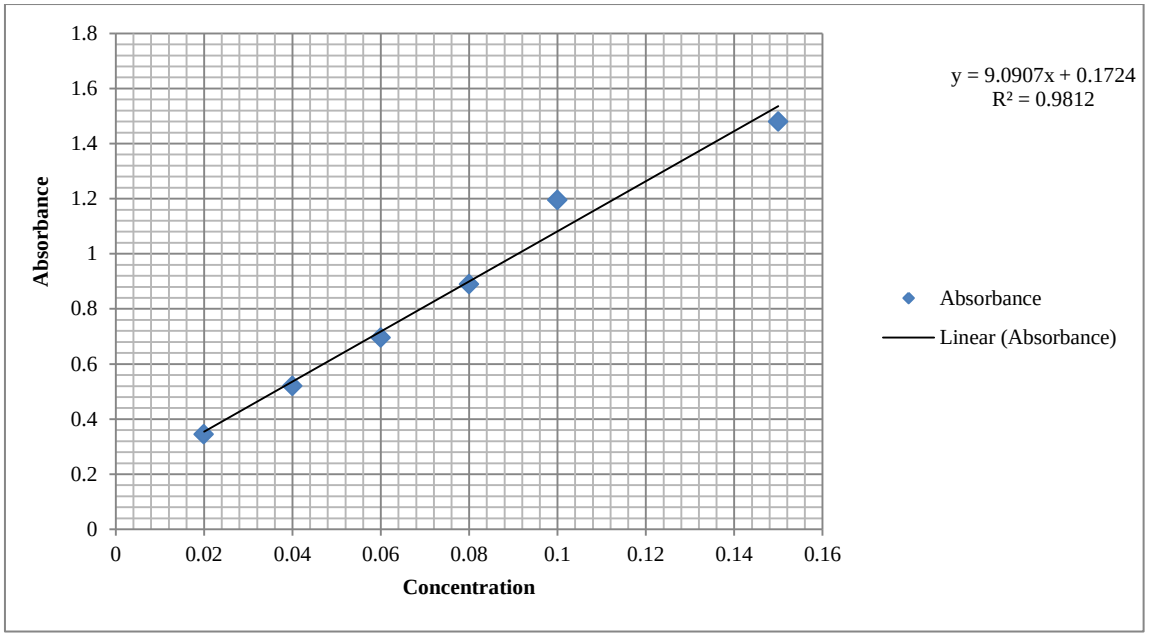

Figure 4: Quercetin calibration curve for the determination of total flavonoids content.

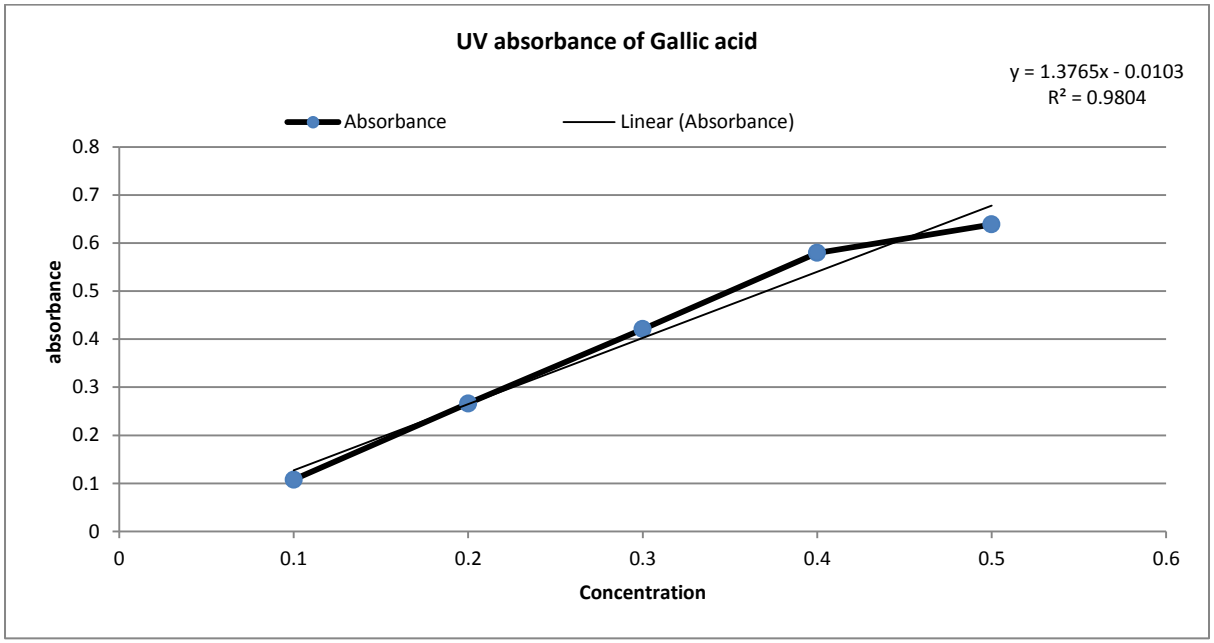

Figure 5: Gallic acid calibration curve for the determination of Total Tannin Content.

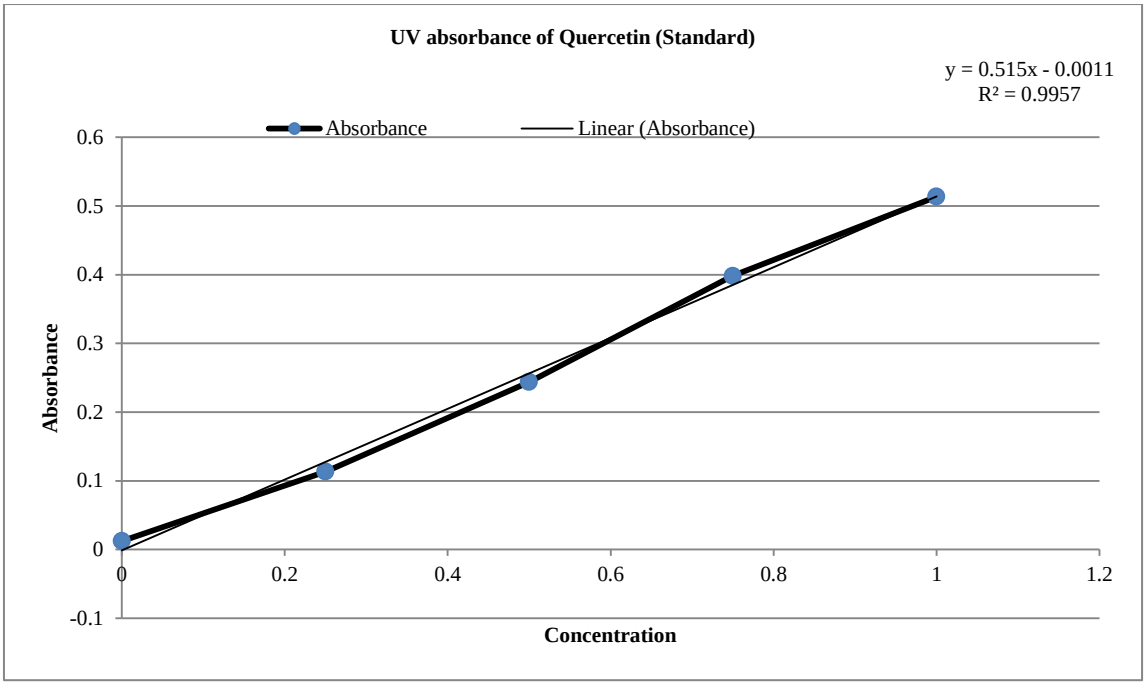

Figure 6: Quercetin calibration curve for the determination of Total Flavonoids Content.

Antibacterial activity test

The antibacterial activity of *B. orellana* extract was performed using two different antibiotics as positive control. At the doses of 250 and 500 µg/disc, the extract showed antibacterial activity against the experimental bacterial strains namely *Shigella dysenteriae, Pseudomonas aeruginosa* (*Pseudomonas aeruginosa* didn't show activity at 250 µg/disc), *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* as compared with Flucloxacillin (30 µg/disc) and Ciprofloxacin (30 µg/disc). The maximum zone of inhibition was found against *Staphylococcus aureus* that was 20.5 and 26.5 mm when Flucloxacillin was used as positive control (Table 5 and Figure 7) and 21 and 27 mm when Ciprofloxacin was used as positive control (Table 6 and Figure 8) at the dose of 250 and 500 µg/disc respectively.

Table 5: *In vitro* antibacterial activity of crude extract using Flucloxacillin antibiotic disc as positive control.

Antibacterial activity of crude extract using Standard antibiotic disc	Bacterial Strains								
	S. pyogenes Gram (+)	*S. aureas* Gram (+)	*V. cholera* Gram (-)	*S. dysenteriae* Gram (-)	*P. species* Gram (-)	*S. enteritidis* Gram (-)	*P. aeruginosa* Gram (-)	*S. typhi* Gram (-)	*E. coli* Gram (-)
Blank	0	0	0	0	0	0	0	0	0
Flucloxacillin (30 µg/disc)	36	35	0	0	18	0	30	0	23
Sample (250 µg/disc)	0	20.5	0	16.5	0	0	0	13	12.5
Sample (500 µg/disc)	0	26.5	0	19.5	0	0	23	21.5	18

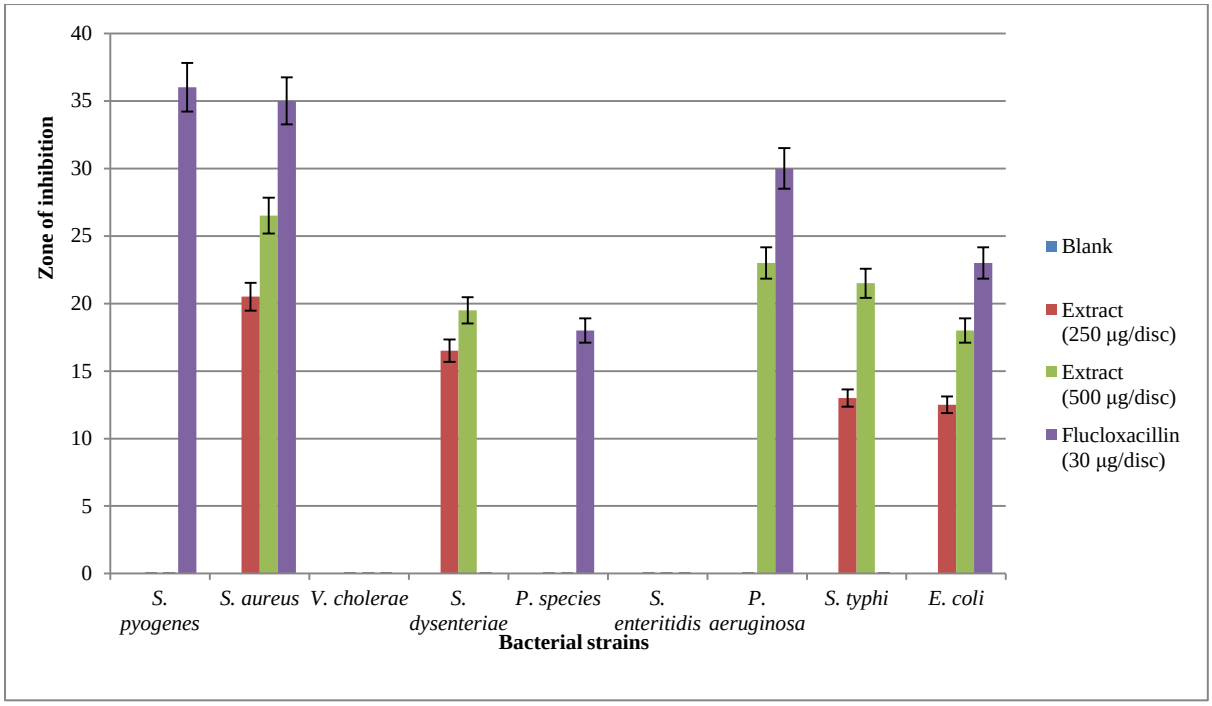

Figure 7: Antibacterial activity of *B. orellana* at two different doses against gram positive and gram negative bacteria in comparison with Flucloxacillin.

Table 6: *In vitro* antibacterial activity of crude extract using Ciprofloxacin antibiotic disc as positive control.

Antibacterial activity of crude extract using Standard antibiotic disc	Bacterial Strains								
	S. pyogenes **Gram (+)**	***S. aureas*** **Gram (+)**	***V. cholera*** **Gram (-)**	***S. dysenteriae*** **Gram (-)**	***P. species*** **Gram (-)**	***S. enteritidis*** **Gram (-)**	***P. aeruginosa*** **Gram (-)**	***S. typhi*** **Gram (-)**	***E. coli*** **Gram (-)**
Blank	0	0	0	0	0	0	0	0	0
Ciprofloxacin (30 µg/disc)	36	35	30	26	26	25	30	35	22
Sample (250 µg/disc)	0	21	0	16	0	0	0	13	12
Sample (500 µg/disc)	0	27	0	20	0	0	22	22	19

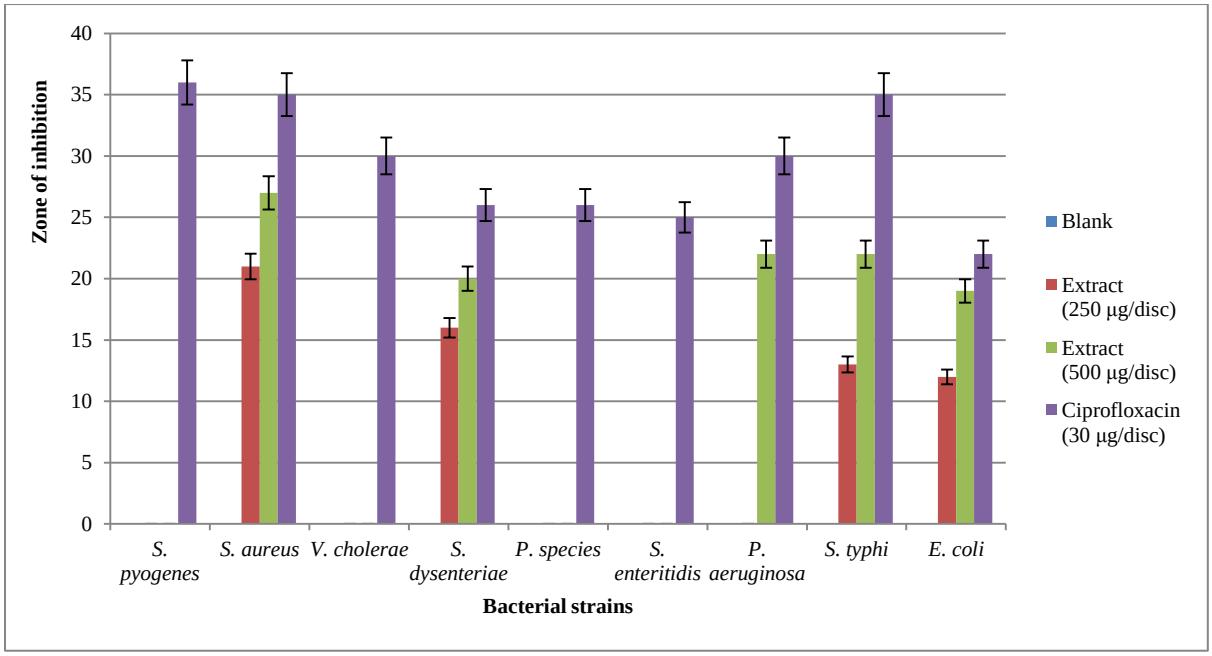

Figure 8: Antibacterial activity of B. orellana at two different doses against gram positive and gram negative bacteria in comparison with Ciprofloxacin.

Analgesic activity test

The ethanol extract of *B. orellana* at the doses of 250 mg/kg and 500 mg/kg body weight exhibited significant inhibition of writhing reflex by 40.34% and 67.05% respectively while the standard drug Diclofenac sodium inhibition was found to be 79.55% at a dose of 25 mg/kg body weight (Table 7 and Figure 9)**.**

Table 7: Effects of the extract at the doses of 250 and 500 mg/kg-body weight.

Treatment	Dose (mg/kg b. wt.)	Mean of writhing	% inhibition
Negative control	--	35 ± 0.86	--
Diclofenac Na	25	7.2 ± 0.58*	79.55
Extract	250	21 ± 0.71*	40.34
Extract	500	11.60 ± 0.51*	67.05

Values are expressed as mean ± SEM (standard error for mean), n = 5, *p< 0.01 vs. control.

Here, b. wt- body weight.

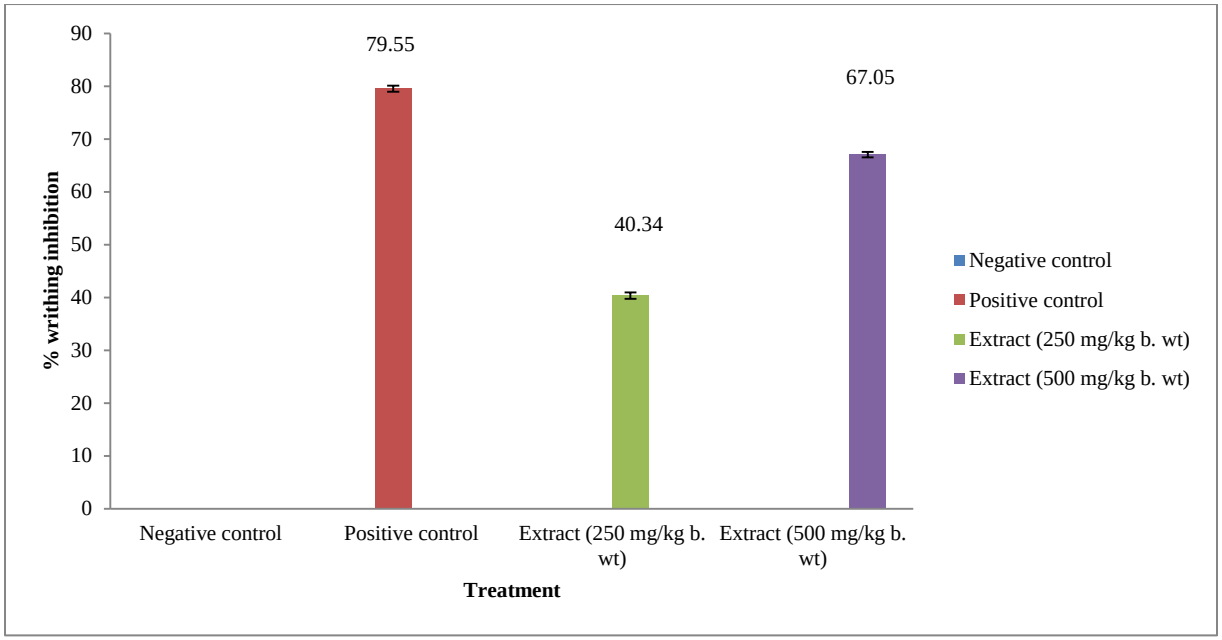

Figure 9: % Writhing inhibition by the standard drug & extract (*** $p < 0.01$).

Anthelmintic activity test

The crude extract of *B. orellana* showed dose dependent decrease in paralysis time and death time of the parasite. The time taken for paralysis and death at 25 and 50 mg/mL concentration was approximately 33 minutes & 39 minutes and 23 minutes & 30 minutes respectively whereas standard showed approximately 7 and 16 minutes at the dose of 15 mg/mL respectively against the parasite (Table 8 and Figure 10).

Table 8: Paralysis time and death time of parasite at different concentration.

Treatment	Conc. (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Negative control	--	--	--
Albendazole	15	7.5 ± 0.18	16.23 ± 0.47
Extract	25	33.16 ± 0.3	39.23 ± 0.41
Extract	50	22.52 ± 0.47	30.08 ± 0.57

Values are expressed as mean ± SEM (standard error for mean), n = 6.

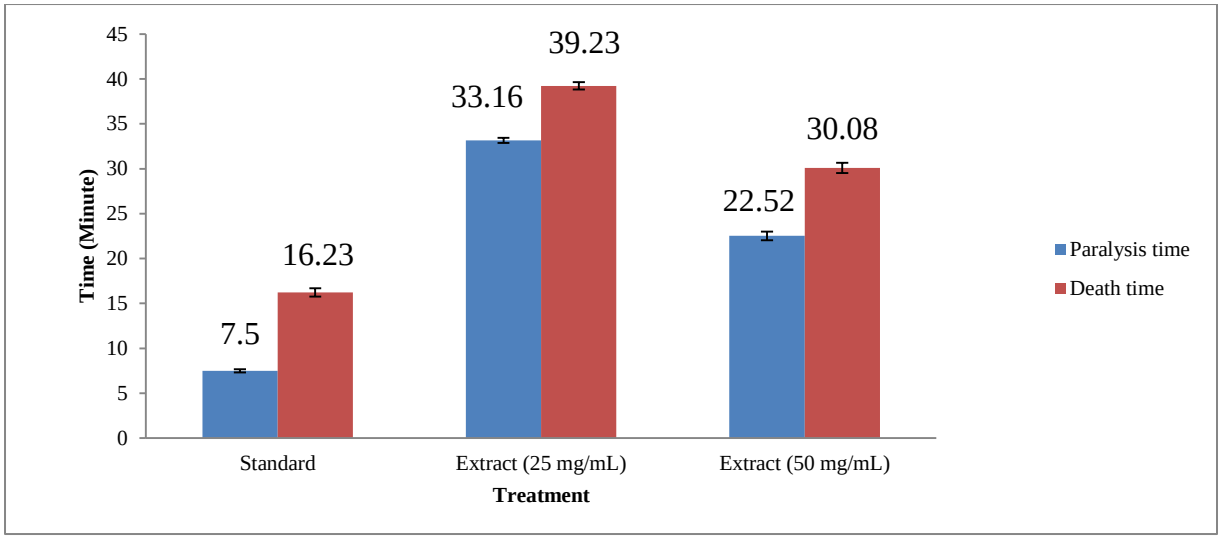

Figure 10: Anthelmintic activity of ethanol extract of seeds of *B. orellana.*

DISCUSSION

Identification of the nature of the compounds present in extract is essential to evaluate the biological activity of the extract. Preliminary phytochemical screening of *B. orellana* seed extract revealed the presence of reducing sugar, flavonoids, tannins, carbohydrates, alkaloids, saponin, proteins, terpenoids, steroids and acidic compounds. Among these constituents phenolic compounds, flavonoids, tannins and alkaloids are the most valuable for therapeutic activity.

The search for antioxidants from natural resources has received much attention and efforts have been made to identify new natural resources for active antioxidant compounds. Antioxidants that scavenge free radicals play an important role in prevention of various disorders [22]. In addition, these naturally occurring antioxidants can be formulated to give neutraceuticals, which can help to prevent oxidative damage occurring in the body [23]. In addition, hydroxyl radicals can reduce disulfide bonds in proteins, specifically fibrinogen, resulting in their unfolding and scrambled refolding into abnormal spatial configurations. Consequences of this reaction are observed in many diseases such as atherosclerosis, cancer and neurological disorders and can be prevented by the action of non-reducing substances [24,25]. There is a plethora of different plants and herbs that contain Polyphenolic substances including simple phenols, phenolic acids, coumarins and isocoumarins, naphthoquinones, xanthones, stilbenes, flavonoids, and lignins can potentially scavenge hydroxyl radicals and/or chelate free iron [26].

It is generally believed that Polyphenolic compounds protect different organs of the body from free radical damage and associated diseases by reducing their amount in the body and also maintain normal human health. Within the antioxidant compounds flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting per-oxidation [26]. The positions of the substituents also affect the physiological properties of different flavonoids. The flavonols having ortho or Para hydroxyl group in the 2- phenyl ring are known to have strong antioxidant properties, while free hydroxyl at the 5, 7-positions proved to have a pro-oxidant effect. On the other hand, tannins have been reported to possess anticarcinogenic and antimutagenic potentials as well as antimicrobial and anti-parasitic properties. The inhibition of lipid peroxidation by tannin constituents can act via the inhibition of cyclooxygenase [27].

For antibacterial activity the phytochemicals like flavonoids, polyphenols and tannins, quinones, terpenoids and essential, alkaloids are essential [28]. Flavonoids show antimicrobial activity by complexing with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Tannins may protect plants from invasion of pathogenic microorganism due to their antimicrobial and antifungal properties. Polyphenols and tannins exert antimicrobial activity through enzyme inhibition, membrane disruption and metal ion

complexation. Alkaloids exert antimicrobial activity through intercalating into cell wall and DNA of parasites [29-31]. *B. orellana* was effective against both gram positive and gram negative bacteria but gram negative bacteria were most sensitive. Phytochemical groups present in *B. orellana* like polyphenols, flavonoids, tannins and alkaloids may be responsible for antibacterial activity.

The phytochemical groups may exert analgesic property by inhibiting the synthesis, release, and/or antagonizing the action of pain mediators at the target sites. The identified phytochemical groups namely flavonoids, tannins, terpenoids, gums and reducing sugar in *B. orellana* seed extract may be responsible for analgesic activity both centrally and peripherally [32-34].

Helminthiasis is a serious disease in human and poultry farming in South-East Asia. Tannins, the secondary metabolite, occurring in several plants have been reported to show anthelmintic property by several investigators [35]. Tannins, the Polyphenolic compounds, are shown to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation or, binds to the glycoproteinon the cuticle of parasite, and cause death. The data presented in the table and observations made thereof, lead to the conclusion that the phytoconstituents, tannin may be responsible for anthelmintic activity.

CONCLUSION

This project work can be summarized into the photochemistry and pharmacology of seeds of *Bixa orellana*. Preliminary phytochemical screening of *B. orellana* seed extract revealed the presence of reducing sugar, flavonoids, tannins, carbohydrates, alkaloids, saponin, terpenoids, proteins, steroids and acidic compounds which are valuable for pharmacological activities. The results of the pharmacological investigation rationalize the uses of seeds of the plant in folk medicine. But more research is needed to find out its individual phytoconstituents in order to introduce this plant part in pharmaceutical industries for developing semi-synthetic and synthetic drugs with similar or better therapeutic properties for the welfare of human being.

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