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In vivo antidepressant, analgesic, anti-inflammatory activities, *in vitro* antioxidant and antibacterial potential of fractionated *Elatostema papillosum* Wed. extract

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Abstract
The study was conducted to evaluated the *in vivo* CNS antidepressant, analgesic, anti-inflammatory, *in vitro* antioxidant and antibacterial activities of crude methanol extract with its petroleum ether soluble fraction (PESF), carbon tetrachloride soluble fraction (CTCSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF) of *Elatostema papilosum* wed. On investigation it was found that crude methanol extract (34 ± 1.76 mins) and its CSF fraction (48 ± 1.13 mins) showed statistically significant (P<0.05) antidepressant activity by increasing phenobarbitone induced sleeping time of mice, produced significant (P<0.05) inhibition in abdominal writhes produced by acetic acid and the degree of inhibition were (24.80 ± 3.32)% and (29.18 ± 2.33)% respectively whereas produced significant (P<0.05) anti-inflammatory effect of (48.13 ± 3.18)% and (54.54 ± 3.78)% respectively after 1st hour of carrageenan injection. It was also observed that methanol extract and its CS fraction showed statistically significant (P<0.01) DPPH free radical scavenging activity with IC_{50} value of 15.14 ± 0.33 µg/ml and 18.45 ± 0.18 µg/ml respectively as well as methanol extract showed 25.1, 21.1, 27.4 and 24.3 mm of diameter of zone of inhibition against four tested gm (+) ve bacteria whereas CS fractions showed 22.3, 24.6, 26.3 and 21.6 mm respectively. Similarly, PES fraction (39 ± 1.80 mins) showed significant (P<0.05) antidepressant activity, as well as significant (P<0.05) anti-inflammatory effect of (40.12 ± 3.75) % after 1st hour of carrageenan injection and showed 26.7 and 24.7 mm of diameter of zone of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords: *Elatostema papilosum*, CNS antidepressant, analgesic, anti-inflammatory, antioxidant

1. Introduction
According to the World Health Report, approximately 450 million people suffer from a mental or behavioral disorder. This amounts of 12.3% of the global burden of disease, and will rise to 15% by 2020 [1]. Depression is most prevalent mental disorder and it is recognized to be symptomatically, psychologically and biologically heterogeneous. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamino oxidase [2], selective serotonin reuptake inhibitors (SSRIs) [2] and selective noradrenalin reuptake inhibitors [2]. Basic neuroscience offers the promise identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drug. This consideration leads to search for new antidepressant agents that have a fast onset of action with fewer side effects [3]. According to the International Association for the Study of Pain (IASP) Pain is defined by as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [4]. Individuals experience pain by various daily hurts and aches, and occasionally through more serious injuries or illnesses. Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants that leads to the local accumulation of plasmatic fluid and blood cells. Inflammation which runs unchecked can lead to a host disease including different type's arthritis such as rheumatoid arthritis, shoulder tendinitis and gouty arthritis [5]. In spite of the progress that has taken place in recent years in the development of therapy, the medical community still urgently needs effective and potent analgesics [9]. This has renewed the interest of plant-derived secondary metabolites as part of the search for new clinically useful drugs. Free radical oxidation is another concerning matter now days. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [6].

Recently plant species have been investigated in the search for novel antioxidants [10] for reducing such free radical induced tissue injury but generally there is still a demand to find more information concerning the antioxidant potential of plant species. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [7, 8].

Elatostema papillosum Wed. belongs to the family of Urticaceae is used in this context as plant of interest. It is a suberect herb, up to 30 cm long. The species commonly occurs in the greater districts of Chattogram and Chattogram Hill tracts. Medicinally the plant is used in the treatment of cirrhosis, liver cancer, paralysis, rheumatic arthritis, rheumatism and scabies [11]. *E. papillosum* was selected in this investigation because most of the pharmacological effects, bio-active compound isolation, elemental profile and radioactivity analysis this plants still remain unexplored.

With the development in techniques and recent researches, various compounds in plants has been proved to have a varied pharmacological activity such as CNS antidepressant, anti-inflammatory, analgesic, antioxidant and antimicrobial and cytotoxic properties. In this context, this is a matter of interest to evaluate the pharmacological activity like CNS antidepressant, anti-inflammatory, analgesic, antioxidant and antimicrobial properties of different fractionates of *E. papilosum* wed. In experimental animal model.

2. Materials and Methods

2.1 Animals

Swiss albino mice of both sexes weighing between 25 to 30 g and Wistar Albino rats of the either sex weighing between 150-200 g obtained from animal house of BCSIR laboratories, Chittagong were used for the present study. The animals were acclimatized to room temperature (28 ± 5 °C) with a relative humidity of (55 ± 5) % in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study, the animals were supplied with standard pellet diet and water *ad libitum.* In this study, all the animal experimentation was carried out according to the guidelines of Institutional Animal Ethics Committee (IAEC).

2.2 Preparation of crude extract and its different fractionates

Whole plant parts of *E. papillosum* Wed. Were collected from Chattogram hill tracts of Bangladesh. The plant was taxonomically authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Professor of Department of Botany, University of Chittagong. The plants were sun dried for several days and then oven dried for 24 hours at considerably low temperature (below 40 °C) to facilitate grinding. The powdered material (500 g) was macerated in 2.5 L of methanol for 15 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40 - 45 °C) and reduced pressure. The concentrated methanol extract (ME) was partitioned by the modified Kupchan method [12] and the resultant fractionates i.e., petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions were used for the experiment.

2.3 CNS antidepressant activity

Phenobarbitone induced Sleeping test was carried out according to the method of Williamson *et al.*, (1996) [13]. The test animals were divided in seven groups consisting of five mice per group. Group I was the control group whereas group II, III, IV, V, VI and VII were experimental groups. The experimental groups were administered with test samples prepared with normal saline water and Tween-80 at doses of 400 mg/kg body weight, while the control group was administered normal saline water containing 1% Tween-80 solution. Thirty minutes later Phenobarbitone sodium (25 mg/kg body weight) was administered intraperitoneally to all the groups to induce sleep. The onset of sleep and total sleeping time were recorded for both control and treated groups.

2.4 Analgesic activity

Acetic acid induced writhing test model as described by Koster *et al.*, (1959) was performed to evaluate the analgesic activity [14]. The test animals were divided in seven groups consisting of five mice per group. Group I was the control group, group II was the positive control group received standard diclofenac sodium (10 mg/kg) whereas group III, IV, V, VI and VII were experimental groups. 1% (v/v) acetic acid solution (3.3 ml/kg body weight) was injected intraperitoneally in mice and the number of writhing and stretching was counted over 20 minutes period. Methanol extract with it different fractionates (2 g/kg), reference analgesic drug diclofenac sodium (10 mg/kg) and distilled water were administered orally to respective groups, 30 min before acetic acid injection. Finally, percent (%) analgesic activity was calculated by using following formula-

$$\% \text{ Analgesic activity} = \frac{\{\text{Mean writhing count (Control group - Treated group)} \times 100}{\text{Mean writhing count of control group}}$$

2.5 Anti-inflammatory activity

Carrageenan induced paw edema a model as described by Winter *et al.*, (1962), was used for evaluating potential of methanol extract and it different fractionates of *E. papilosum* on inflammation [15]. The test animals were divided in seven groups consisting of five mice per group. Group I was the control group, group II was the positive control group received standard diclofenac sodium (10 mg/kg) whereas group III, IV, V, VI and VII were experimental groups. The initial right hind paw volume of each rat was measured using plethysmometer (UGO Basile, Italy) and then 0.1 ml of 1 % (w/v) carrageenan was subcutaneously injected into the sub-plantar region of the right hind paw in order to induce acute inflammation. The volume of right hind paw was measured at 1st, 2nd, 3rd and 4th hour after carrageenan injection and the paw edema was determined using plethysmometer. *E. papilosum* methanol extract and its different fractionates (2 g/kg), standard anti-inflammatory drug diclofenac sodium (10 mg/kg) and distilled water were administered orally to respective groups one hour before the sub-plantar injection of carrageenan. The inhibitory activity was calculated according to the following formula:

$$\% \text{ of inhibition} = \frac{\{(Ct - Co) \text{ Control group} - (Ct - Co) \text{ Treated group}) \times 100\}}{(Ct - Co) \text{ Control group}}$$

Where Ct is the right hind paw thickness volume (in mm^3) at time t, Co is the right hind paw thickness volume (in mm^3) before carrageenan injection. Ct - Co is paw edema, (Ct - Co) control is edema or paw size after carrageenan injection to control rats at time t. (Ct - Co) treated is edema or paw size after carrageenan injection to treated (reference or sample drug) rats at time t.

2.6 *In vitro* antioxidant activity

The antioxidant activity was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity according to the method described by Brand-William *et al.*, (1995) with slight modifications [16]. Methanol extract of *E. papilosum* with different fractionates (0.2 mg/ml) and standard ascorbic (0.2 mg/ml) acid were prepared in methanol. DPPH solution was prepared in methanol and 3 ml of this solution was mixed with 1 ml of extract solution and standard solution separately. These solution mixtures were kept in dark for 30 min. The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. The absorbance of the mixture was determined at 517 nm using UV-Visible Spectrophotometer (Cintra, Australia) and ascorbic acid was served as a positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The scavenging activity against DPPH was calculated using the following equation:

Scavenging activity (%) = [(A - B) / A] x 100

Where A was the absorbance of control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid). Then, % scavenging activity or % inhibition was plotted against log concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis.

2.7 Antibacterial activity

Antibacterial activity of plant extracts was determined by disc diffusion method as described by Bauer *et al.*, (1966) [17]. In this study 4 Gram (+) ve and 6 Gram (-) ve bacterial strains were used. A measured amount of methanol extract with its different fractionates (400 µg/disc) and Ciprofloxacin (30 µg/disc) as standard antibiotic were used in the present study. The bacteria cultures were grown in Nutrient Broth (NB) medium at 37 ºC. After 6 h of growth, each microorganism, at a concentration of 10^7 cells/ml, was inoculated on the surface of Mueller-Hinton agar plates. Dried filter paper discs (4 mm in diameter) were then impregnated with known amounts of test substances (400 µg/disc) by using micropipette. Discs containing the test material were then placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Discs soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4 ºC) for two to four hours to allow maximum diffusion of compound. The plates were then incubated at 37 ºC for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called "Zone of Inhibition". The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

3. Results

3.1 Assay of CNS antidepressant activity

In this investigation it was observed that methanol extract, CS and PES fraction of *E. papilosum* showed statistically significant (P<0.05) antidepressant activity compared to control (30 ± 1.19 minutes) by increasing phenobarbitone induced sleeping time of mice like 34 ± 1.76 minutes, 48 ± 1.13 minutes and 39 ± 1.80 minutes respectively. Here all the values are expressed as Mean ± SEM.

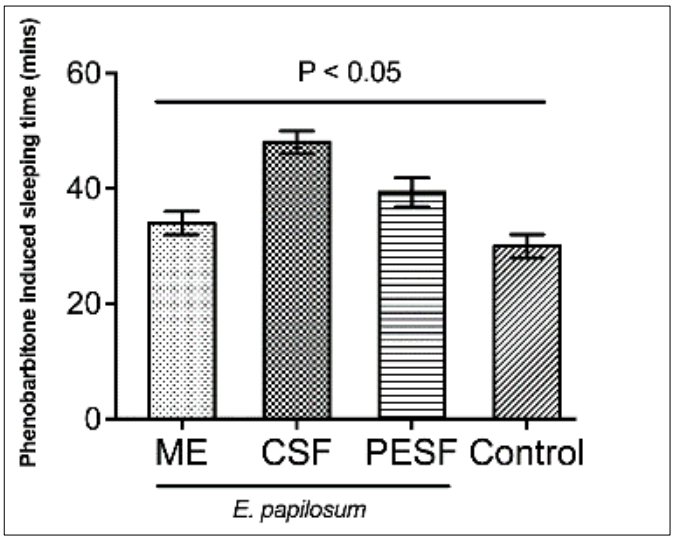

Fig 1: Antidepressant activity of statistically significant fractionates of *E. papilosum* on mice

3.2 Assay of analgesic activity

Methanol extract and CS fractions of *E. papilosum* showed statistically significant (P<0.05) analgesic activity in acetic acid induced writhing test model on Swiss albino mice. Methanol extract showed (24.80 ± 3.32)% and CS fraction showed (29.18 ± 2.33)% of analgesic activity compared to standard diclofenac sodium which showed (45.23 ± 4.23)% of analgesic activity respectively. All the values are expressed as Mean ± SEM.

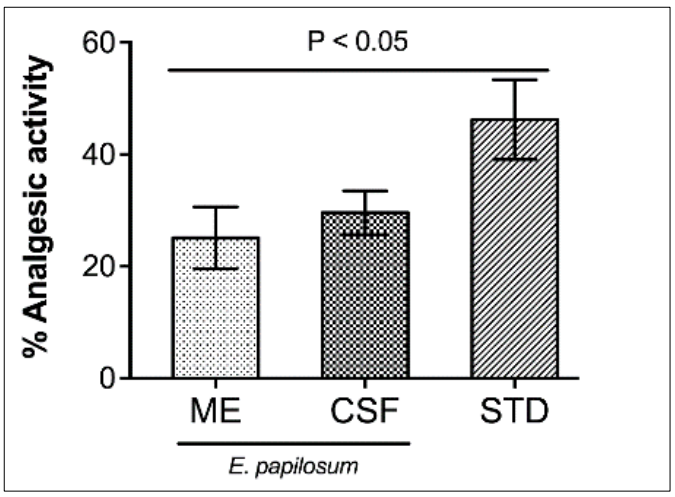

Fig 2: Analgesic activity of statistically significant fractions of *E. papilosum* on mice

3.3 Assay of anti-inflammatory activity

Methanol extract, PES and CS fractions of *E. papilosum* inhibited significant paw edema of rats produced by carrageenan which means that the mentioned fractions

showed statistically significant (P<0.05) anti-inflammatory activity. Carrageenan was subcutaneously injected into the sub-plantar region of the right hind paw of rats and the volume of right hind paw was measured at 1st, 2nd, 3rd and 4th hour after carrageenan injection using plethysmometer. Methanol extract, PES fraction and CS fraction inhibited inflammation of rats paw after 1st hour were (48.13 ± 3.18)%, (40.12 ± 3.75)% and (54.54 ± 3.78)% respectively compared to standard diclofenac sodium (70.31 ± 2.34)%. All the values are expressed as Mean ± SEM.

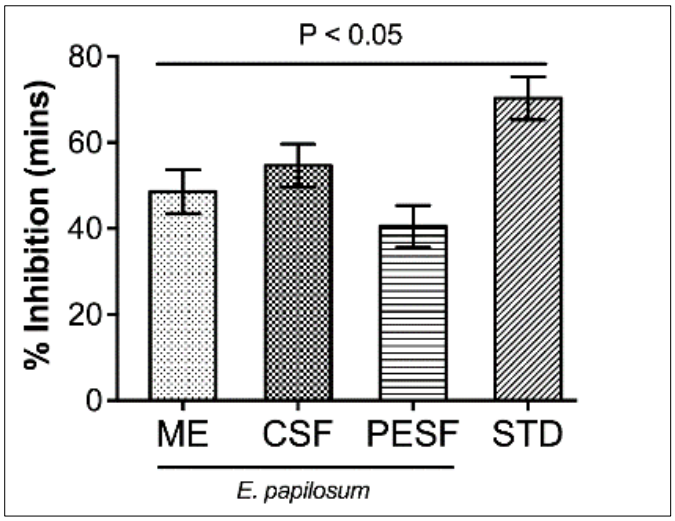

Fig 3: % Inhibition of paw edema by different fractionates of *E. papilosum* on rats

3.4 Assay of antioxidant activity

Methanol extract and its CS fraction of *E. papilosum* showed statistically significant (P<0.01) free radical scavenging activity by inhibiting free radical formation compared to standard ascorbic acid. IC_{50} values found for methanol extract and CS fraction of *E. papilosum* were 15.14 ± 0.33 and 18.45 ± 0.18 respectively compared to standard ascorbic acid (10.12 ± 0.48). Here all the values are expressed as Mean ± SEM.

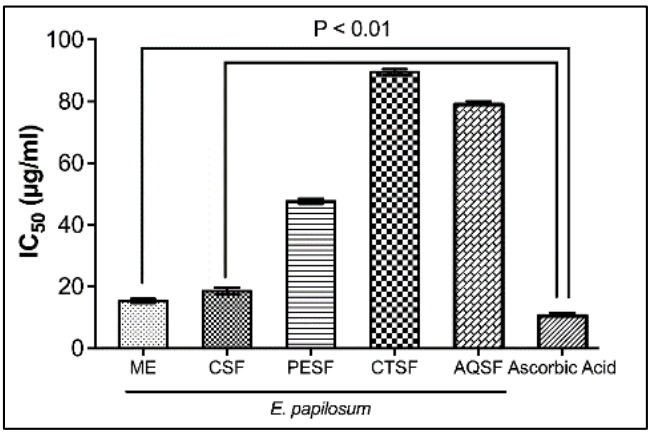

Fig 4: DPPH free radical scavenging activity of different fractionates of *E. papilosum* in terms of IC_{50} value (µg/ml) compared to standard ascorbic acid

3.5 Assay of antibacterial activity

Antibacterial activity against gm (+) ve and gm (-) ve bacteria have been determined by disc diffusion method. Here Methanol extract and CS fractions showed significant zone of inhibition in petri plate against gm (+) ve bacteria whereas PES fraction of *E. papilosum* showed moderate activity against *B. subtilis* and *S. aureus* and all fractionates showed insignificant activity against gm (-) ve bacteria.

Table 1: Zone of inhibition showed against gm (+) ve and gm (-) ve bacteria by different fractionates of *E. papilosum* compared to standard ciprofloxacin

Bacterial Type	Test Microorganisms	Diameter of Zone of Inhibition (mm)					
		ME	PESF	CTCSF	CSF	AQSF	Ciprofloxacin
Gram (+) ve	*Bacillus cereus*	25.1	16.5	5.7	22.3	---	**47.3**
	Bacillus megaterium	21.1	17.5	5.3	24.6	---	43.3
	Bacillus subtilis	27.4	26.7	4.6	26.3	8.4	45.6
	Staphylococcus aureus	24.3	24.7	1.2	21.6	---	48.3
Gram (-) ve	*Escherichia coli*	12.4	13.6	---	13.4	---	46.3
	Pseudomonas aeruginosa	15.7	14.6	----	14.6	8.9	43.6
	Slmonella paratyphi	17.4	12.4	---	10.3	---	45.6
	Slmonella typhi	12.7	11.2	2.8	16.7	---	48.6
	Shigella dysenteriae	13.5	13.1	-----	13.8	---	49
	Vibrio cholera	14.1	10.2	1.4	11.6	3.9	45.3

4. Discussion

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. A vast majority of our population, particularly those living in villages depend largely on herbal remedies. A good number of herbal remedies have stood the test of time particularly for the treatment of allergic, metabolic and degenerative diseases associated with aging. However, very few scientific data regarding their identity and effectiveness of these herbs was available except that in the treatise of Ayurveda and Unani medicine. In this study, we have conducted various *in vivo* and *in vitro* experiments to assess the CNS antidepressant, analgesic, anti-inflammatory, antioxidant and antibacterial effect of the methanol extract with its different fractionates like petroleum ether soluble fraction (PESF), carbon tetrachloride soluble fraction (CTCSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF) of *E. papillosum.*

Investigation of CNS antidepressant activity was determined by phenobarbitone induced sleeping time method. We observed that methanol extract, CS and PES fraction of *E. papilosum* showed statistically significant (P<0.05) antidepressant activity by increasing phenobarbitone induced sleeping time of mice like 34 ± 1.76 minutes, 48 ± 1.13 minutes and 39 ± 1.80 minutes respectively compared to control (30 ± 1.19 minutes). CTC and AQS fraction showed insignificant activity. The evaluation of analgesic activity of the plant was carried out by acetic acid induced writhing

response model. We found that the treatment of animals with methanol extract and CS fraction of *E. papilosum* produced significant (P<0.05) inhibition in abdominal writhes produced by acetic acid and the degree of inhibition of methanol extract and CS fractions were (24.80 ± 3.32)% and (29.18 ± 2.33)% respectively comparable to standard analgesic drug diclofenac sodium (45.23 ± 4.23)%. PES, CTC and AQS fraction showed insignificant analgesic activity. Anti-inflammatory activity of *E. papilosum* was carried out using carrageenan induced paw edema model. Methanol extract, PES fraction and CS fraction produced anti-inflammatory effect of (48.13 ± 3.18)%, (40.12 ± 3.75)% and (54.54 ± 3.78)% respectively after 1st hour of carrageenan injection compared to standard diclofenac sodium (70.31 ± 2.34 %). And significant (P<0.05) anti-inflammatory effect were observed in all phases of the experiment except for CTC and AQS fraction. Antioxidant activity of methanol extract of *E. papilosum* was assessed by DPPH free radical scavenging activity. In this study, the DPPH scavenging effect of extracts was compared with standard antioxidant ascorbic acid. Methanol extract and its CS fraction of *E. papilosum* showed statistically significant (P<0.01) free radical scavenging activity by inhibiting free radical formation compared to standard ascorbic acid with IC_{50} value of 15.14 ± 0.33 µg/ml and 18.45 ± 0.18 µg/ml respectively compared to standard ascorbic acid (10.12 ± 0.48 µg/ml). PES, CTCS and AQS fraction showed statistically insignificant DPPH free radical scavenging activity. Methanol extract showed 25.1, 21.1, 27.4 and 24.3 mm of diameter of zone of inhibition against four tested gm (+) ve bacteria like *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilus* and *Staphylococcus aureus* whereas CS fractions showed 22.3, 24.6, 26.3 and 21.6 mm respectively. And PES fraction showed 26.7 and 24.7 mm of diameter of zone of inhibition against *B. subtilis* and *S. aureus* and all fractionates showed insignificant activity against gm (-) ve bacteria.

From phytochemical study of different plants we know that components like phenolics (Carvacol, Curcumin), polyphenolic flavonoids (Proanthocyanidin) and amino acids (L-Theanine, Ferulic acid) is responsible for CNS antidepressant activity whereas steroid, phenolics and flavonoids is responsible for analgesic and anti-inflammatory activity of the plant. Again components like phenolics, flavonoids and carotenoids are the cause of antioxidant activity whereas alkaloids, tannins and quinones are responsible for antibacterial activity [18]. So, some of these compounds might be involved behind the observed CNS antidepressant, analgesic, anti-inflammatory, antioxidant and antibacterial activities of *E. papilosum*.

5. Conclusion and future plan

To the best of our knowledge, this is the first report describing pharmacological activities of *E. papilosum.* However, further studies are necessary to elucidate the mechanism behind these effects. As previously no data regarding this plant published so we may say that *E. papilosum* may be an exemplary sample for alternative therapeutic source of drugs. In this regards, we have to elucidate the structure of secondary metabolites present in the plant and give emphasis on novel compound as therapeutic component. This report may serve as a footstep on this aspect.

6. Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

7. Acknowledgements

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8. Author contributions

M.Z.U., T.B.E., M.D., S.M.A.U., S.H. and M.S.R. performed experiments. M.Z.U., T.B.E. and M.D. performed the antidepressant, analgesic and anti-inflammatory activity. M.Z.U. and S.M.A.U. performed the antibacterial activity. M.Z.U., T.B.E. and S.M.A.U. performed the statistical analysis. M.Z.U., T.B.E., M.D., S.M.A.U., S.H. and M.S.R. conceived the study and designed the experimental procedures. M.S.R. and S.H. supervised the study. M.Z.U. and T.B.E. wrote the manuscript. All authors read and approved the manuscript.

9. References

1. Drag G, Vifen T. Mental health: new understanding new hope. J world health rpt. 2001; 14:03-211.
2. Reynolds EH. Brain and mind: a challenge for WHO. Lancet. 2003; 361:1924-1925.
3. Thase ME, Howland RH. Biological process in depression: an update and integration. Handbook of depression. New York.
4. Pasero C, McCaffery M. Pain: clinical manual. St. Louis: Mosby, 1999. ISBN 0-8151-5609-X.
5. Underwood JCE. General and systematic pathology. Edn 3, Churchill Livingstone, New York, 2000, 201-201.
6. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry, UK, 1999; 178-187.
7. Jansen AM, Cheffer JJC, Svendsen AB. Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of test methods. Planta Med. 1987; 40:395-398.
8. Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW. Antimicrobial constituents of Rhus glabra. J Ethnopharmacol. 1994; 42:95-99.
9. Calixto JB, Scheidt C, Otuki M, Santos AR. Biological activity of plant extracts: novel analgesic drugs. Expert Opinion on Emerging Drugs. 2001; 6(2):261-279.
10. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2-diphenyl-picryl-hydrazyl radical. African J Biomed. Res. 2002; 5:77-79.
11. Uddin SN. Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts. Implemented by Bangladesh National Herbarium. 541-541.
12. Muhit MA, Tareq SM, Apu AS, Basak D, Islam MS. Isolation and Identification of Compounds from the Leaf Extract of *Dillenia indica* Linn. Bangladesh Pharmaceutical Journal. 2010; 13(1):49-53.
13. Williamson EM, Okpako DT, Evans FJ. Pharmacological Methods in Phytotherapy Research: Selection, preparation and pharmacological evaluation of plant material. Edn 1, John Willey & Sons, England, 1996.
14. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Federation Proceeds. 1959; 18: 412-416.
15. Winter CA, Risley EA, Nuss CW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;

111:544-547.
16. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol. 1995; 28:25-30.
17. Bauer AW, Kirby WM, Sheriss JC, Turc M. Antibiotic susceptibility testing by standardized single method. Am. J Clin. Pathol. 1996; 45:493-496.
18. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A review. Int. Pharmaceutical Science. 2011; 1(1):98-106.