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DECIPHERING THE PHARMACOLOGICAL INSIGHTS OF FRACTIONATED ELATOSTEMA PAPPILLOSUM WED. AND HOLIGARNA LONGIFOLIA ROXB. THROUGH IN VITRO AND IN VIVO STUDIES

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KEYWORDS

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ABSTRACT

The present research intended to explore the biological activities, namely acute toxicity test and hypoglycemic as well as *in vitro* anti-arthritis along with the antibacterial activity of crude methanol extracts with its different soluble fractions like petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous soluble fraction (AQSF) of *Holigarna longifolia* and *Elatostema papillosum*. Phytochemical screening was performed by established protocols. Acute toxicity and hypoglycemic effects were performed in experimental and alloxan-induced diabetic rats. *In vitro* anti-arthritis and antibacterial activity were conducted by protein denaturation inhibitory and disc diffusion methods. It was observed that no rats exhibit any lethality types, which reveal the safety of plant fractionates. It was also seen that both plants' fractionates showed significant ($p < 0.01$) activity on hyperglycemia compared to standard. Upon investigation, it was observed that crude methanol and its CS fraction of *E. papillosum* and only CS fraction of *H. longifolia* significantly ($p < 0.05$) inhibited denaturation of bovine serum albumin protein compared to standard diclofenac sodium. Moreover, it was observed that crude methanol extract and its CS fraction of *E. papillosum* showed significant inhibitory action on all Gram-positive bacteria's growth. In contrast, the PES fraction highlighted an inhibitory zone of 26.7 and 24.7 mm, respectively, towards *B. subtilis* and *S. aureus*. This study provides some support to explain the traditional uses of *H. longifolia* and *E. papillosum*.

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1 Introduction

World Health Organization (WHO) says, about 80% of the world population specially in developing countries are mostly depend on the traditional medicine from plants for their healthcare (Rahman et al., 2013; Biswas et al., 2014) in the form of tea, decoction or extracts with water, milk (or) alcohol (Bristy et al., 2020) and WHO has also recommended to use plants as medicines where the conventional medicine is not easily available (Rakib et al., 2020). The holistic practice of medicine like Ayurveda, homeopathy, siddha, unani are in practice of using the plant herbal formulations to treat various ailments (Uddin et al., 2019). Diabetes is one of the most prevalent metabolic disorders characterized with elevated level of blood glucose and inappropriate metabolism. Insulin is released by the pancreas which utilizes blood glucose into the cells for energy production. Without presence of insulin glucose level gets elevated in blood and soon after eliminated through the urine. Diabetes also causes different types of additional problems like retinopathy, neuropathy and nephropathy. These problems commonly originate due to DNA damage caused by free radical generation (Tareq et al., 2020). It is also associated with high heart risks caused by means of improper cholesterol metabolism which in turn leads to hyperlipidemia (O'Brien et al., 1998; Shifah et al., 2020) and hence it is necessary to search for a molecule which can lowers blood glucose level, scavenges free radical and decrease hyperlipidemia. This need prompted us the current investigation. In the modern world, human beings are affected by numerous metabolic diseases such as diabetes, cancer, arthritis, gastrointestinal diseases etc. Among the metabolic diseases, one of disease affecting most of the people is arthritis. Likewise, arthritis is one of the oldest diseases and it a systemic inflammatory disease which mainly affects the joints (Subramoniam et al., 2013). The usual age of onset of arthritis is between 25 and 50 which occur more frequently in the people of age group 40 s and 50 s (Patwardhan & Hopper, 1992; Yesmin et al., 2020).

The most commonly used drugs in the conventional modern medicine are non-steroidal anti-inflammatory drugs (NSAIDS) which shows potent injurious effects like stomach irritation, malfunction of the kidney, urticaria, liver disorders, hematological abnormalities and gastrointestinal problems which includes ulcers, bleeding, heartburn, diarrhea, retention of fluid and perforation of stomach or intestine (Yesmin et al., 2020). Rheumatoid arthritis is a chronic disease characterized by the hyperactive state of some specific reactions related with immune system, chronic synovitis and in most cases, it causes deposition of rheumatoid factor (RF) an auto antibodies to self-antigens (Lipsky, 2008; Akter et al., 2020). It affects about 1% of world population (Silman & Pearson, 2002) and the prevalence of RA is highly in adult men of age between 30-50 years. The prevalence of RA in women are about 0.8%, it is more prevalent in women than men. The conventional

modern medicine is just aimed at reducing the pain, inflammation, damage to articular structure etc. but it is not totally curative. Based on the traditional information many of the plant extracts (or) and active fractions were tested in experimental animal models of arthritis and inflammation.

Infectious diseases are major causes of morbidity and mortality in the developing world and accounts for about 50% of all deaths (Emran et al., 2015). Wide ranges of plants were extensively used for multidimensional diseases. These benefits provide form their big content on bioactive compounds (Cheruvanky, 2004; Kumar et al., 2013). With the development in techniques and recent researches, various compounds in plants has been proved to have a varied pharmacological activity such as neuroprotective, anti-diabetic, anti-inflammatory, anti-arthritic, antinociceptive, antioxidant, antidiarrhoeal, antimicrobial, and cytotoxic properties. In this context, this is a matter of interest to evaluate the pharmacological properties like antibacterial, anti-arthritic, antidiabetic activities and toxicity study of different fractionated extracts of *H. longifolia* and *E. papillosum*.

2 Materials and Methods

2.1 Plants Sample

Holigarna longifolia Roxb. (locally known as Borola/Katebel), a member of Anacardiaceae family and *Elatostema papillosum* Wed., a member of Urticaceae were used in this context as plants of interest. Both plant species occurs in the forest areas of Sylhet, Chattogram and Chattogram Hill tracts. Traditionally *H. longifolia* is used for the healing of nasal polyps whereas *E. papillosum* is used in the treatment of cirrhosis, liver cancer, paralysis, rheumatic arthritis, rheumatism and scabies (Uddin, 2002; Uddin et al., 2019). These plants were chosen for the current study as majority of their pharmacological properties, isolation of phytoconstituents as well as elemental profile exploration still remains in unexplored condition.

2.2 Preparation of Crude Extract and It's Different Fractionates

Leaves of *E. papillosum* and *H. longifolia* were collected from Chittagong Hill tracts area. Both plants were taxonomically authenticated by Department of Botany, University of Chittagong. Methanol extracts of both plants were extracted through cold extraction process by following standard procedures with some modifications by using rotary vacuum evaporator at a temperature of (40 - 45 °C) under compact pressure. Concentrated methanol extracts (ME) of both plants were partitioned by the modified Kupchan method (Muhit et al., 2010) and the resultant soluble fractionates i.e., PESF, CTCFSF, CSF and AQSF were isolated.

2.3 Phytochemical Group Analysis

Phytochemical group tests were done to ensure the presence of secondary metabolic constituents which was identified by characteristic color changes using standard procedures described by Ghani, Sofowara, Trease and Evans and Harborne (Ghani, 2003; Trease & Evans, 1989; Sofowara, 1993; Harborne, 1973).

2.4 Experimental Animals

Wistar Albino rats of both sex of 150-200 g weight were employed in this research. The rats were nurtured in a wire meshed plastic cages prior to commencement of the experiment for 4 to 5 days at ambient temperature (28 ± 5 °C). During the regimen of experiment, recommended pellet diet with water *ad libitum* were given to the rats as normal diet. Animals were handled and maintained in accordance with the guiding principle provided by Institutional Animal Ethics Committee of the Faculty of Biological Science, University of Chittagong, Bangladesh (AERB/FBS/UC/02, 2015).

2.5 Acute Toxicity Test

Acute toxicity study has been performed through the method described by Harizal et al. (2010). *In vivo* acute toxicity study was employed by 423 guiding principles (Acute toxicity class method) set down by OECD (Organization of Economic Cooperation and Development). Albino rats with sound physical state were indiscriminately alienated into eight classes where each class had six (6) rats. The animals were reserved fasting for the night with plenty of water, thereafter with extracts of *E. papillosum* and *H. longifolia* with increasing doses (100, 200, 300, 400, 500, 600, 700, and 800 mg/kg body weight) with the aid of intragastric tube in order to determine the safe doses by up and down staircase method.

2.6 Anti-diabetic Activity

Hypoglycemic effects of both plants fractionates have been investigated on diabetic rats by the method prescribed by Kannur et al. (2006).

2.6.1 Experimental Design

A total of 138 male albino Wistar rats were employed and arbitrarily separated into 23 sets of six (n=6) rats in every set:

Group I: Normal control (untreated with dimethyl sulfoxide [DMSO, 3 ml/kg]).

Group II: Diabetic control (alloxan administered).

Group III: Diabetic control + Glibenclamide (0.5 mg/kg body weight once a day orally for 10 days).

Group IV: Diabetic control + *E. papillosum* methanol extract.

Group V: Diabetic control + *E. papillosum* PES fraction.

Group VI: Diabetic control + *E. papillosum* CTS fraction.

Group VII: Diabetic control + *E. papillosum* CS fraction.

Group VIII: Diabetic control + *E. papillosum* AQS fraction.

Group IX: Diabetic control + *H. longifolia* methanol extract.

Group X: Diabetic control + *H. longifolia* PES fraction.

Group XI: Diabetic control + *H. longifolia* CTS fraction.

Group XII: Diabetic control + *H. longifolia* CS fraction.

Group XIII: Diabetic control + *H. longifolia* AQS fraction.

Group XIV: Normal rats receiving *E. papillosum* methanol extract.

Group XV: Normal rats receiving *E. papillosum* PES fraction.

Group XVI: Normal rats receiving *E. papillosum* CTS fraction.

Group XVII: Normal rats receiving *E. papillosum* CS fraction.

Group XVIII: Normal rats receiving *E. papillosum* AQS fraction.

Group XIX: Normal rats receiving *H. longifolia* methanol extract.

Group XX: Normal rats receiving *H. longifolia* PES fraction.

Group XXI: Normal rats receiving *H. longifolia* CTS fraction.

Group XXII: Normal rats receiving *H. longifolia* CS fraction.

Group XXIII: Normal rats receiving *H. longifolia* AQS fraction.

Oral doses of 400 mg/kg for 3 hours for all extracts were used in this experiment. The extracts were administered to the respective groups through oral route using intragastric tube for 3 hours.

2.6.2 Induction of Experimental Diabetes and Treatment

An intraperitoneal solution of Alloxan monohydrate (10 mg/ml) was induced in the rats within the interval of 5 mins at a dose of 50 mg/kg body weight. Ice-cold citrate buffer of 0.1 M of pH 4.5 was used for preparing the solution of alloxan. Alloxan was chosen to induce diabetes due to its availability and widely reported in previous research (Soto et al., 1994; Doss & Dhanabalan, 2008). After 48 h of administration, diabetic model rats with hyperglycemia and glycosuria were taken for the experiment.

Table 1 Presence of phytochemicals in crude methanol extracts of *E. papillosum* and *H. longifolia*

Serial No.	Secondary metabolites	Name of the species	
		<i>E. papillosum</i>	<i>H. longifolia</i>
1	Alkaloids	+	-
2	Flavonoids	+	+
3	Steroids	+	+
4	Tannins	+	-
5	Saponins	+	+
6	Phlobatannins	-	-
7	Glycosides	+	+

“+” indicates presence of secondary metabolites whereas “-” denotes the absence of secondary metabolites

Table 2 Hypoglycemic actions of different fractionates of *E. papillosum* and *H. longifolia* compared to standard glibenclamide

Sample	Hypoglycemic Activity (mmol/L)							
	<i>E. papillosum</i> (400 mg/kg)				<i>H. longifolia</i> (400 mg/kg)			
	Initial	60 min	120 min	180 min	Initial	60 min	120 min	180 min
ME	15.5± 1.23	13.5± 1.58	12.0± 1.23	11.3± 1.91	16.8± 1.45	12.9± 1.24	12.2± 1.31	11.2± 1.55
PESF	14.4± 1.76	14.4±1.23	13.3± 1.44	12.1±1.51	16.4±1.67	12.7± 1.08	12.1±1.12	11.3± 1.25
CTCSF	16.8± 1.67	13.5±1.76	12.8± 1.14	12.3±1.57	16.1± 1.12	13.4± 1.19	12.3± 1.31	11.3± 1.23
CSF	16.2± 1.79	12.2± 1.31	11.9± 1.49	10.6±1.33	16.7± 1.71	13.0± 1.54	12.7± 1.33	11.2± 1.35
AQSF	15.9± 1.32	11.1±1.12	10.9± 1.47	9.3± 1.11	15.7± 1.14	13.5± 1.13	12.8± 1.56	12.3± 1.67
Control (1% Tween-80 solution in saline)					16.7± 1.34	6.8± 1.67	6.2± 1.12	5.6± 1.32
Standard (5 mg/kg glibenclamide)					16.3±1.42	3.4± 1.21	2.6± 1.21	2.5± 1.78

SEM = standard error of mean, n = 5. Values in the table are articulated as mean ± SEM, *p < 0.05, significantly different in comparison with standard. The data was analyzed by ANOVA followed by Dunnett's test

Table 3 Statistical analysis of hypoglycemic effects of different fractionates of *E. papillosum* and *H. longifolia* compared to standard glibenclamide

Code no	<i>E. papillosum</i>		<i>H. longifolia</i>	
	p-value	Level of significance	p-value	Level of significance
STD	0.0214	Statistically significant	0.0214	Statistically significant
ME	0.9345	Statistically not significant	0.885	Statistically not significant
PESF	0.7687	Statistically not significant	0.6672	Statistically not significant
CTCSF	0.7364	Statistically not significant	0.7241	Statistically not significant
CSF	0.6431	Statistically not significant	0.6998	Statistically not significant
AQSF	0.5451	Statistically not significant	0.6412	Statistically not significant

2.6.3 Hypoglycemic Activity Test

Diabetic rats were reserved fasted for 8 hours. After accomplishment of fasting stage, fasting blood glucose (FBG) level of the rats were estimated by glucometer kit (Clever Check, Germany). Consequently, diabetes was introduced by intraperitoneal administration of alloxan at a dose of (70 mg/kg) (Aruna et al., 1999). Blood was drawn after 1h, 2h and 3h consecutively from each rat by tail snipping for determining diabetic blood glucose. Animals with blood glucose level ≥ 10 mmol/dl were marked as hyperglycemic and used for the study.

2.7 In vitro Anti-arthritis Activity

Following subsequent steps were maintained for the evaluation of anti-arthritis activity (i) 0.5 ml of test solution, control solution and product control were made of 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (250 $\mu\text{g/ml}$), 0.05 ml of distilled water along with 0.05 ml of test solution (250 $\mu\text{g/ml}$) respectively (ii) Similarly 0.5 ml of standard solution was made with 0.45 ml of Bovine serum albumin and 0.05 ml of diclofenac sodium (250 $\mu\text{g/ml}$). All solutions were accustomed to pH 6.3 with 1N HCl. All solutions were kept at 37 °C for 20 minutes and heat was augmented to 57 °C for 3 minutes. Subsequent to cool, 2.5 ml of phosphate buffer was added to all of the solutions. Finally optical density was measured by UV-Visible spectrophotometer at the wavelength of 416 nm (Mizushima & Kobayashi, 1968; Aruoma, 1998). The percent (%) of protein denaturation inhibition can be expressed as,

$$\text{Percent protein denaturation inhibition} = \frac{100 - (\text{absorbance of test solution} - \text{optical density of product control})}{\text{optical density of test control}} \times 100$$

Here control stands for 100% denaturation of protein.

2.8 Antibacterial Activity

Antibacterial activity of plant extracts was evaluated by disc diffusion method explained by Bauer et al. (1966). In this study four Gram +ve and six Gram -ve bacterial strains were used. A measured amount of crude extract, different fractionates (400 $\mu\text{g/disc}$) and Ciprofloxacin (30 $\mu\text{g/disc}$) as standard antibiotic were used in the present study. The bacteria cultures were nurtured in Nutrient Broth medium at 37 °C. After 6 hours, culture of every microorganism was inoculated on the plane of Mueller-Hinton agar plates. Filter paper discs of 4 mm in diameter were then soaked with esteemed quantity of test substances (400 $\mu\text{g/disc}$) by using micropipette. Discs of test sample were then sited on agar plate medium unvaryingly germen with the experimental microorganisms. Discs drenched in relevant solvent were marked as positive control. The plates were then reserved at 4 °C temperature for two to four hrs to permit highest distribution of molecule. The experimental plates were subsequently kept in 37 °C

for 24 hours to allocate utmost expansion of tested microorganisms. A clear distinct zone around the disc reflects the antibacterial activity of test materials.

2.9 Statistical Analysis

Statistical investigation was evaluated with the help of Graph Pad Prism software package, version 7.0. All the statistics were articulated as mean \pm standard error mean (SEM). The comparisons within groups were evaluated utilizing independent student t-test and one-way analysis of variance (ANOVA). The values of $p < 0.05$ or $p < 0.01$ were regarded as statistically significant.

3 Results

3.1 Screening of Phytochemicals

From phytochemical screening it was observed that *E. papillosum* contains alkaloids, tannins, saponins, glycosides and higher percentage of flavonoids and steroids whereas *H. longifolia* contains flavonoids, steroids, glycosides and higher percentage of saponins. The results of table 1 showed the presence of phytochemicals in crude methanol extracts of *E. papillosum* and *H. longifolia*.

3.2 Acute Toxicity Study

Acute toxicity study was employed on healthy male albino rats. The animals were reserved fasting for the night with plenty of water supply, thereafter with extracts of *E. papillosum* and *H. longifolia* with increasing doses (100, 200, 300, 400, 500, 600, 700, and 800 mg/kg body weight) with the aid of intragastric tube in order to determine the safe doses. No lethality and death was observed which indicates that both plant extracts were showed no toxicity in rats

3.3 Anti-diabetic Activity

All fractionated extracts of both plants were administered serially to all 23 groups of rats and then diabetic level was measured after 1st, 2nd, and 3rd hour using glucometer. It was observed that all fractionates showed no hypoglycemic effects on alloxan induced diabetic rats. Here all the statistics were presented as mean \pm SEM (standard error mean). Table 2 and 3 presents the results of anti-diabetic activity.

3.4 Anti-arthritis Activity

In this investigation it was observed that crude methanol and its CS fraction of *E. papillosum* showed statistically significant ($p < 0.05$) and only CS fractions of *H. longifolia* showed statistically significant ($p < 0.05$) anti-arthritis activity compared to standard diclofenac sodium.

Table 4 Percent inhibition of denaturation of protein by different fractionates of *E. papillosum* and *H. longifolia* compared to standard diclofenac sodium

Sample	Concentration (mg/ml)	% of Inhibition of protein denaturation			
		<i>E. papillosum</i>	p-value	<i>H. longifolia</i>	p-value
ME	200	54.34 ± 2.34*	0.038	23.54 ± 2.32	0.065
PESF	200	29.45 ± 1.34	0.057	18.67 ± 1.17	0.059
CTCSF	200	21.24 ± 3.67	0.076	12.23 ± 1.59	0.097
CSF	200	46.48 ± 2.43*	0.029	38.18 ± 2.78*	0.043
AQSF	200	12.3 ± 2.95	0.094	13.87 ± 2.63	0.091
Diclofenac-Na	50	74.93 ± 1.12			

SEM = standard error of mean, n = 5. Values in the table are articulated as mean ± SEM, *p < 0.05, significantly different in comparison with standard. The data was analyzed by ANOVA followed by Dunnett's test

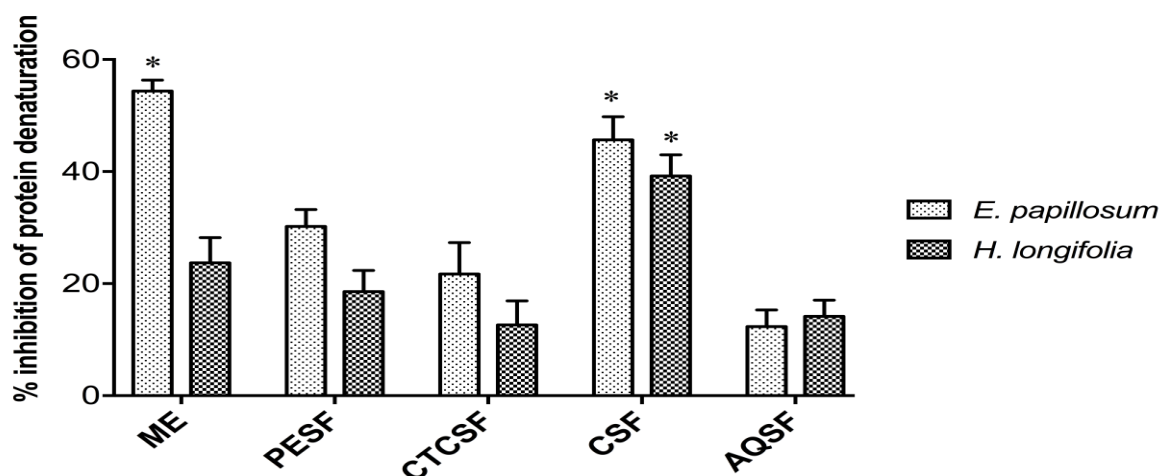


Figure 1 Comparison of the activity of different fractionates of *E. papillosum* and *H. longifolia* on the percentage of inhibition of protein denaturation

Here all the values are articulated as mean ± SEM. The results are given in Table 4. Figure 1 represents the comparison of the activity of different fractionates of *E. papillosum* and *H. longifolia* on the percentage of inhibition of protein denaturation.

3.5 Antibacterial Activity

Antibacterial activity against gm (+) ve and gm (-) ve bacterial type have been carried out through 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. papillosum* showed moderate activity towards *B. subtilis* and *S. aureus* and all fractionates exhibited poor activity towards gm (-) ve bacterial type. But all the fractionates of *H. longifolia* showed poor activity against all gm (+) ve bacteria and exhibited poor activity towards all gm (-) ve bacterial type

compared to standard ciprofloxacin. The results are showed in table 5.

4 Discussion

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. The majority of our population, particularly those living in villages, depends largely on herbal remedies. A good number of herbal remedies have stood the test of time, particularly for treating allergic, metabolic and degenerative diseases associated with ageing. However, very few scientific data regarding their identity and effectiveness of these herbs were available except that in the treatise of Ayurveda and Unani medicine (Ahmed et al., 2019; Banu et al., 2020; Barua et al., 2020; Uddin et al., 2021).

Table 5 Zone of inhibition showed against gm (+) ve and gm (-) ve bacterial type by different fractionates of *E. papillosum* and *H. longifolia* compared to standard ciprofloxacin.

Bacterial Type	Gram +ve type						Gram -ve type				
	<i>B. cereus</i>	<i>B. megaterium</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. paratyphi</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>V. cholera</i>	
<i>(E. papillosum)</i>	ME	25.1	21.1	27.4	24.3	12.4	15.7	17.4	12.7	13.5	14.1
	PESF	16.5	17.5	26.7	24.7	13.6	14.6	12.4	11.2	13.1	10.2
	CTSF	5.7	5.3	4.6	1.2	---	---	---	2.8	---	1.4
	CSF	22.3	24.6	26.3	21.6	13.4	14.6	10.3	16.7	13.8	11.6
	AQSF	---	---	8.4	---	---	8.9	---	---	---	3.9
<i>(H. longifolia)</i>	ME	10.3	12.4	13.2	18.8	3.6	5.4	3.7	5.8	4.8	5.2
	PESF	6.2	8.5	9.3	10.2	5.3	4.9	6.4	3.5	3.7	5.4
	CTSF	---	2.8	3.3	---	---	---	2.7	2.2	---	---
	CSF	11.5	16.8	15.9	19.8	10.1	9.2	14.4	10.7	14.9	10.8
	AQSF	9.1	---	10.8	---	---	---	---	---	6.92	4.7
Diameter of Zone of Inhibition (mm) for Standard Ciprofloxacin		47.3	43.3	45.6	48.3	46.3	43.6	45.6	48.6	49	45.3

In this study, we have conducted various *in vivo* and *in vitro* experiments to assess the acute toxicity study, anti-diabetic, anti-arthritis and antibacterial activities of 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*, and *H. longifolia*.

Although phytochemicals obtained from medicinal plants were focused on the empiric experience in the past, currently, the scientific evidence regarding the chemical composition and therapeutic properties are regarded as the main focus while isolating several phytochemicals. Expert taxonomists mainly perform the identification and authentication of medicinal plants; however, one of the main disadvantages includes the absence of

several phenotypic characteristics. Besides, the products used in traditional medicine are processed in various forms, such as powder, extracts, capsules, and tablets. Therefore, phytochemical characterisation could be used to identify and authenticate several medicinal plants (Dutta et al., 2019; Banu et al., 2020; Jahan et al., 2020).

Nowadays antibiotic resistance is a major challenge in the healthcare sector of the world. Multidrug resistant pathogens have significantly endangered the present antibacterial therapy. This urgency has committed us to search a new source of antimicrobial drugs like plants as they contain variety of bioactive phytochemicals. This study has been conducted to evaluate the antibacterial activity of two medicinal plant extracts against human pathogens (Romero et al., 2005; Boucher et al., 2009).

Investigation of antibacterial activity was carried out with the help of disc diffusion method and it was observed that crude methanol extract and its CS fraction of *E. papillosum* showed significant zone of inhibition towards all gm (+) ve bacteria where as PES fraction exhibited 26.7 and 24.7 mm of zone of inhibition towards *B. subtilis* and *S. aureus*. But all fractionates of *H. longifolia* showed poor activity against all gm (+) ve bacteria and exhibited poor activity towards all gm (-) ve bacteria compared to standard ciprofloxacin. The difference in activities may be due to the use of different solvent system (Mohan et al., 2016). It has been widely observed and accepted that the different bioactive phytochemicals present in the plants dissolves in different solvent systems (Cowan, 1999).

The protein-denaturation study for determining anti-arthritis activity was performed using bovine serum albumin (BSA). Upon heating BSA exhibits denaturation and antigens are expressed which are linked with type-III hypersensitivity reaction, that leads to some autoimmune diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness and systemic lupus erythematosus (Kishore et al., 2011). Protein denaturation leads to production of autoantigen which is one of the important causes of rheumatoid arthritis. Denaturation may be due to the alteration of electrostatic, hydrogen, hydrophobic and disulfide bonds (Arya et al., 2014). All the extracts had a dose-dependent response in the *in vitro* anti-arthritis test. It was observed that crude methanol ($p = 0.038$) and its CS fraction ($p = 0.029$) of *E. papillosum* and only CS fraction ($p = 0.043$) of *H. longifolia* significantly ($p < 0.05$) inhibited denaturation of bovine serum albumin compared to standard diclofenac sodium. The promising activities of the extracts support their uses as remedies for arthritis, rheumatism and other chronic inflammatory conditions (Elisha et al., 2016).

Before going to *in vivo* studies acute toxicity study on rats were done to determine whether the fractionated extracts are safe or not. Here both plant fractionates was done by applying higher doses (100, 200, 300, 400, 500, 600, 700, and 800 mg/kg body weight) of all fractionates of both plants on experimental rats and it was observed that no rats exhibit any types of lethality or abnormalities which reveals about the safety of plant fractionates.

Researchers who work with diabetes and related disease are still trying to develop a safer medicines for diabetes (Banagar et al., 2013). The development of modern treatment methods requires animal models that mimic the range of pathophysiological changes visualized in diabetic humans. Here *in vivo* hypoglycemic activity was performed in alloxan-induced diabetic rats. And it was seen that fractionates of both plant showed insignificant ($p > 0.01$) activity against hyperglycemia in diabetic rats compared to standard glibenclamide which indicates that fractionates of both plants have no hypoglycemic effect against alloxan induced diabetic rats. In, *in vitro* anti-arthritis activity percent inhibition of

protein denaturation was estimated spectrophotometrically. From phytochemical screening it was observed that *E. papillosum* contains alkaloids, tannins, saponins, glycosides and higher percentage of flavonoids and steroids whereas *H. longifolia* contains flavonoids, steroids, glycosides and higher percentage of saponins. Phytochemicals like terpenoids, phenylpropanoids, flavonoids, coumarins, sterols, and alkaloids present in plant extracts show antibacterial activity (Yan et al., 2006; Li et al., 2008), where as presence of steroids and flavonoids are responsible for anti-arthritis activity of the plant (Tiwari et al., 2011). So, some of these phytochemicals might be concerned behind the experimental activities of *E. papillosum* and *H. longifolia*.

Conclusions

To the best of our knowledge, very few data regarding *E. papillosum* and *H. longifolia* has been published. However, additional studies are compulsory to elucidate the mechanism behind these effects. As previously, no data regarding this plant published, so we may say that both plants may be an exemplary sample for an alternative therapeutic source of drugs. In this regard, we have to elucidate the structure of secondary metabolites present in the plants and emphasise novel compounds as therapeutic components. This report may serve as a footstep on this aspect.

Conflict of interest

The authors report no conflicts of interest. The authors only are answerable for the content and inscription of the paper.

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Author's contribution

This exertion was employed in teamwork of all authors. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission. Authors MZU, MSR, SC, and AP performed experiments. Authors MZU and SC collected the plants and prepared the extracts and fractions. MZU and AP performed the anti-arthritis and antibacterial activity. MZU and MSR performed the anti-diabetic activity and hypoglycemic activity. MZU, SC, and AP performed statistical analysis. MZU, MSR, SH, SC, and AP conceived the study and designed the experimental procedures. MSR and SH designed, planned and supervised the experiments. TBE and KD acted for all correspondences. MZU, MSR, SH, and AP participated in the

manuscript draft and has thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. All authors read and approved the final version of the manuscript.

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