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PHYSICOCHEMICAL, PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF LEAVES EXTRACT OF *Mimosa rubicaulis* (Lam).

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ABSTRACT

Herbal plants are an important part of our natural wealth. They are the foremost therapeutic agents as well as valued raw materials for manufacturing various traditional and modern medicines. Organoleptic evaluation helps in the identification and authentication of the crude drug and also in detecting adulterants. It is based on stimuli received by the five organs of sense-odor or smell, taste, color, and how it is perceived, touch or feel, and sound. Physicochemical analyses were performed using WHO-recommended parameters, and fluorescence behavior of the leaf samples was also analyzed. The phytochemical analysis was carried out on the leaves of the *Mimosa rubicaulis* (Lam) plant extracted with increasing polarities of solvent like petroleum ether, Chloroform, ethyl acetate, ethanol, and aqueous solvents. The Proximate values for leaf of *Mimosa rubicaulis* (Lam) is majorly seen in Alcohol-soluble extractive value 10.32%. The qualitative analysis showed that contains tannins, glycosides, in some amount of alkaloids and carbohydrate, a major amount of Flavonoids and triterpene were seen in ethanol and ethyl acetate solvent. Quantitative Phytochemical Analysis carried out for the determination of Carbohydrates, Saponins, Alkaloids, and Flavonoids. Amongst them Flavonoids were seen largely in leaf extraction with ethanol 3.33 (w/w) and ethyl acetate 2.32 (w/w) solvent. The presence of a high amount of phytochemical compounds suggests that the *Mimosa rubicaulis* (Lam) plant has higher medicinal value and can be extensively studied to extract the natural compounds which are beneficial to human beings and that could be commercialized for higher production than using synthetic drugs with fewer side effects.

Keywords: *Mimosa rubicaulis* (Lam), Phytochemical, Qualitative analysis, Quantitative analysis, Flavonoids

INTRODUCTION

In recent years, several scientific investigations of ancient seasoning remedies for many diseases are disbursed and this has led to the development of other drug and therapeutic ways. Since the consumption of meditative plants is increasing, it's attention-grabbing to use these plants as a supplement in food taking into consideration that these plants will gift a big quantity of trace components and different nutrients**[1-4]**. it's necessary to spot that bioactive constituent of meditative plants typically utilized by herbalists within the treatment of infectious diseases. *Mimosa rubicaulis* (Mimosaceae) mostly found in open sandy places, the arid zones of Rajasthan, Punjab, Delhi, Central, and South India. Herbal medicine is a scientifically recognized complementary and alternative treatment method with proven efficacy.A time-honoured system of healing practiced in every culture in the world. Science has modernized the system using analytical and pharmaceutical testing. The science-based practice of herbal medicine is now called phytomedicine or phytotherapy, which is a system of therapeutics in which diseases and disorders are treated with medicinal plants and preparations made from them using scientific principles **[5]**. *Mimosa rubicaulis* (Lam) are widely used in treatment of urinary complaints, applied to burns, the powdered root is given for vomiting, over glandular swelling and also used in dressing for sinus, sores and leaves infusion is used in piles. Smoke rising from burning the gum is used as a disinfectant**[6]**.

MATERIALS AND METHODS

Collection and Authentication of Plant:-

Fresh & healthy plant parts of *Mimosa rubicaulis* (Lam) like leaf were collected in a separate sterile polythene bags from Wildlife Institute of India, Dehradun. Collected plant parts were examined and identified with the help of regional floras the plants were identified and authenticated at ICMR-National Institute of Traditional, Department of Health Research, Ministry of Health & Family welfare, Govt. of India, Belgam with accession numbers RMRC-1446 was done.

Macroscopical and Microscopical evaluation:-

The Macroscopical or morphological identification characters like shape, size, fracture, surface features, etc. These give the identification of the crude drug as well as the morphological group to which it belongs **[7]**. Sometimes characters of the family or genus may also be found by this method. The morphological terminology is derived from botany and zoology, depending upon the source of the drug. In general, colour, odour, taste, size, shape, and the special features of the crude drugs

are to be studied under morphology. The images were taken using a digital micro image adapter with SAGLO software (SAGLO Research Equipment's, India). Randomly selected micro particles for their shape were observed. The micro particles were viewed at a magnification of × 25.

Microscopical is a definite method that helped in the identification of crude drugs, substitutes, and adulterants in addition to macroscopical evaluation. In a group of drugs containing the same chemical compound. Microscopy of different sections of the drug in a particular morphological group of drugs, the Microscopical or the histological characters are mostly similar but with a few differences. These differences form the basis for the identification of the crude drug. The Fine Powder was mounted in glycerine and Stained with iodine, Phloroglucinol + Conc. HCl and Sudan III. Presently the study of powders is gaining importance in pharmacopeia's, as it is simple, quick, and needs very little of the drug to find tissues of diagnostic value **[8, 9]**.

Physicochemical Screening:

Proximate analysis

Proximate values such as moisture content, total ash value, acid insoluble ash, water-soluble ash, alcohol soluble extractive values, and water-soluble extractive values were determined for the powdered drug.

Fluorescence Evaluation:

Some chemical substances absorb light waves of one wavelength and emit visible waves of greater wavelength. So material under observation appears of one colour by ordinary light and of an entirely different colour by ultraviolet light the materials are known as fluorescent and the phenomenon is known as fluorescence.Fluorescence study was carried out with extracts with different solvents (Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water) **[10, 11, 12]**.

Phytochemical investigation:-

Plant Extraction:-

The leaves of the *Mimosa rubicaulis* (Lam) were washed, air dried and later dried at room temperature for seven days. The dried leaves have been powder the use of mortar and pestle **[13]**. Then take powdered plant materials 500 gram turned into additionally filled within the thimble and extracted successively with petroleum ether, chloroform, ethyl acetate ethanol and aqueous solvents in Soxhlet extraction unit for 48 hours **[14]**. The plant extracts were filtered after

which concentrated using rotary evaporator at 40 ºC and every extract had been transferred to glass vials and saved at 4ºC before use **[15]**. Yield of the extract received became calculated

by components as mentioned below:

Extractive yield (%) =Weight of extract / Weight of dried powder ×100

Qualitative phytochemical analysis [**16-23**]

A phytochemical analysis was performed for the extract according to the standard methods described by Brain and Turner (1975) and Evans (1996).

Test for Alkaloid: -

The extracts have been dissolved in diluted hydrochloric acid and filtered. The filtrates were used to evaluate the presence of alkaloids.

Mayer test: The filtrates treated with the Mayer reagent. The formation of a precipitate of yellow cream indicates the presence of alkaloids.

Wagner test: the filtrates treated with the Wagner reagent. The formation of brown / reddish precipitate indicates the presence of alkaloids.

Dragendroff's test

To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent were brought. A prominent reddish brown precipitate shows a positive test.

Test for Carbohydrate: -

Molisch's test: -

2 ml of filtrate, two drops of α-naphthol alcohol solution have been brought. The aggregate became stirred well and 1 ml of focused sulfuric acid become slowly brought alongside the sides of the take a look at tube, the take a look at tube changed into cooled in ice water and left to rest. A pink ring Observe on the hyperlink of two liquids suggests the presence of carbohydrates.

Barfoed's test -

1 ml of filtrate, 1 ml of Barfoed's reagent were added and heated in a water bath for two minutes. The purple precipitate indicates the presence of sugar.

Evidence from Benedict: -

0.5 ml of filtrate become added 0.5 ml of Benedict reagent. The combination was heated in a water bath for two minutes. A colored precipitate suggests the presence of sugar.

Test for Flavonoid: -

Lead acetate test: -

A small amount of extract changed into dissolved in distilled water and 3 ml of 10% lead acetate solution was delivered to it. A voluminous white precipitate suggests the presence of phenolic compounds.

Alkaline reagents: -

An aqueous extract solution changed into handled with a 10% ammonium hydroxide solution; yellow fluorescence suggests the presence of Flavonoids.

Shinoda or Magnesium test -

Hydrochloric acid reduction: -

A small quantity of extract was dissolved in alcohol and a few fragments of magnesium and conc. Hydrochloric acid (drop with the aid of drop) become introduced. If a pink or

crimson red shade develops, the presence of flavonol glycoside is concluded.

Test for Glycoside: -

For the detection of glycoside, about 50 mg of extract were hydrolyzed with concentrated acid for two hours during a water bath, filtered and therefore the hydrolysate was subjected to the subsequent tests.

Legal Test: -

About 50 mg of pyridine extract was dissolved. The sodium nitroprusside solution was added and made alkaline employing a 10% caustic soda solution. The presence of glycoside is indicated by a characteristic pink color.

Borntrager's test: -

2 ml of filtered hydrolysate was added, 3 ml of chloroform was added and stirred, the chloroform layer was separated and a tenth ammonia solution was added. The pink formation indicates the presence of anthroquinone glycosides.

Liebermann - Burchard Test: -

The extract was dissolved in anhydride, heated to a boil, cooled then 1 ml of concentrated vitriol was added along the side of the tube. The red, pink or purple color the fluid junction indicates the presence of steroids / triterpenoids and their glycosides.

Test for phytosterols and triterpenoids: -

Salkowoski test: -

A few drops of concentrated vitriol are added to the agitated and permanent red extract within the lower layer which indicates the presence of steroids and therefore the golden yellow color which indicates the presence of triterpenoids.

Liebermann - Burchard Test: -

The extract was dissolved in anhydride, heated to a boil, cooled then 1 ml of concentrated vitriol was added along the side of the tube. The red, pink or purple color the fluid junction indicates the presence of steroids / triterpenoids and their glycosides.

Saponin detection: -

Foam / foam test: A small amount of the extract was diluted with water to twenty ml. The suspension was stirred during a graduate for quarter-hour. A two centimeter foam or a stable foam layer for 10 minutes indicates the presence of Saponin.

Quantitative phytochemical analysis

Estimate of alkaloids Determination of alkaloids by Harborne, (1973).

One gram of the sample was weighed during a 250 ml beaker and 200 ml of 10% ethanolic acid in ethanol was added and covered and allowed to face for 4 hours. it had been filtered and therefore the extract was concentrated during a water bath to 1 / 4 of the first volume. Conc.NH_4OH was added slowly to the extract until precipitation was Observe. The entire solution was left to settle and therefore the precipitate was collected and

washed with diluted NH_4OH then filtered. The residue is that the alkaloid, which has been dried and weighed **[24]**.

Flavonoid estimate

One gram of plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at temperature. The mixture was filtered through Whatman No1 paper during a pre-weighed 250 ml beaker. The filtrate was transferred to a water bath and allowed to evaporate to dryness and weighed **[25]**.

Estimation of total phenols

The fat-free sample was boiled with 50 ml of ether for extraction of the phenolic component for quarter-hour. Five ml of the extract were pipetted into a 50 ml flask, then 10 ml of water was added. Additionally, two ml of NH_4OH solution and 5 ml of concentrated alcohol were added.

Saponin Determination: -

The samples were ground. 20 g of every plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated during a predicament bath for 4 hours with continuous stirring at about 55 ° C. The mixture was filtered and therefore the residue was extracted again with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml during a water bath at about 90 ° C. The concentrate was transferred to a 250 ml separating funnel and 20 ml of ether were added and shaken vigorously. The aqueous layer was recovered while the ethereal layer was discarded. The purification process was repeated. 60 ml of n-butanol were added. The combined extracts of n-butanol were washed twice with 10 ml of aqueous common salt. The remaining solution was boiled during a water bath. After evaporation, the samples were dried during a constant weight oven. The Saponin content was calculated as a percentage **[26]**.

Carbohydrate Estimate: -

100 mg of sample were hydrolysed during a boiling tube with 5 ml of 0.5 N HCl during a boiling water bath for a period of three hours. It had been cooled to temperature and solid washing soda was added until the effervescence ceased. The content was centrifuged and therefore the supernatant was made at 100 ml using water. From this, 0.2 ml of sample was pipetted and therefore the volume was delivered to one ml with water. Then one ml of phenol reagent then 5.0 ml of vitriol was added. The tubes were kept at 25-30 ° C for 20 minutes. Absorbance was read at 490nm **[27]**.

RESULTS

Organoleptic and Macroscopic evaluation

Organoleptic study the bodies of Plant such as roots, stems, leaves, and flower parts comprised of the bodies of higher plants as

seen in **(Table 1)**The powder of the dried herb of *Mimosa rubicaulis* is dark green with a characteristic bitter odor and taste. The Organoleptic characteristics of the plant are summarized below.

Microscopical evaluation:-

The microscopic evaluation method allows a more detailed examination of a drug and it can be used to identify organized drugs by their known histological characters. Microscope, by its property to magnify, permits the minute structure understudy to be enlarged and can be used to confirm the structural details of the drugs from plant origin. T.S of the leaf shows trichomes, stomata, and the upper and lower epidermis.

Physicochemical Screening:

Proximate analysis

The result of proximate analysis shown in **(Table 2) and (Graph 1)** shown alcohol soluble extractive value is Maximum Proximate Value.

Fluorescence Evaluation:

The fluorescence of extracts of different solvents helps in the determination of the quality and purity of the drug **(Table 3)**.

Phytochemical investigation:-

Plant Extraction:-

In **(Table 4)** shown the percentage of extraction in different Solvent.

Qualitative phytochemical analysis

The present study was carried out on the *Mimosa rubicaulis* (Lam.) revealed that the presence of active phytochemical constituents. Qualitative estimation in **(Table 5)** indicated the presence of Secondary metabolites like alkaloids, glycoside, Flavonoids, Saponin, Carbohydrate, and some amount of Phytosterols and triterpenoids in the plant extract.

Quantitative phytochemical analysis

Quantitative estimation in **(Table 6)** indicated a major quantity of secondary metabolite present in different extract compare to another extract.

Table 1: Organoleptic features of *Mimosa rubicaulis* (Lam)

Sr.No.	Characteristics	Leaf
1	Colour	Yellowish green
2	Shape	Thin, flat, curved
3	Size	Leaves are double-compound, 8–15 cm long, with thorny rachis. Leaves have 3-12 pairs of side-stalks, each with 6-15 pairs of tiny oblong leaflets 4–8 mm
4	Features	-
5	Odor and Taste	Pungent, bitter and astringent

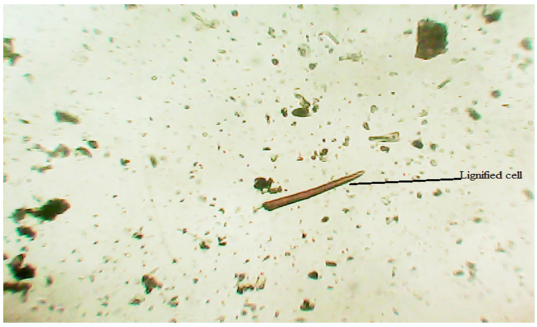

Figure 1:Ligfied Cell

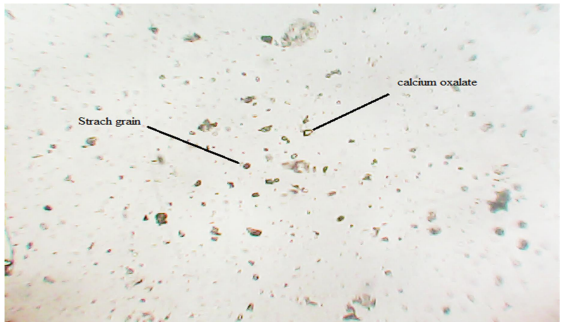

Figure 2: Starch grain and Calcium Oxalate crystal

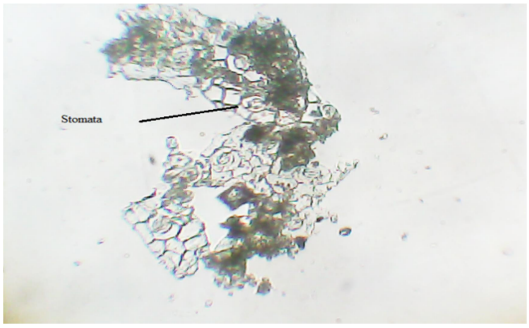

Figure 3: Stomata

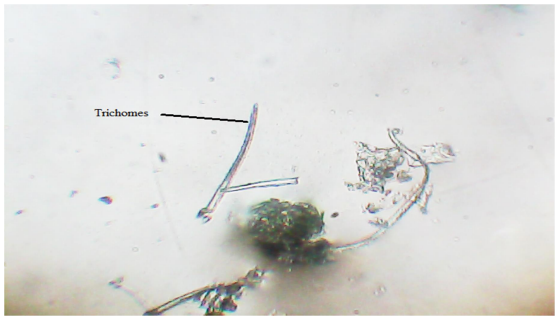

Figure 4: Unicellular curved, warty covering trichomes

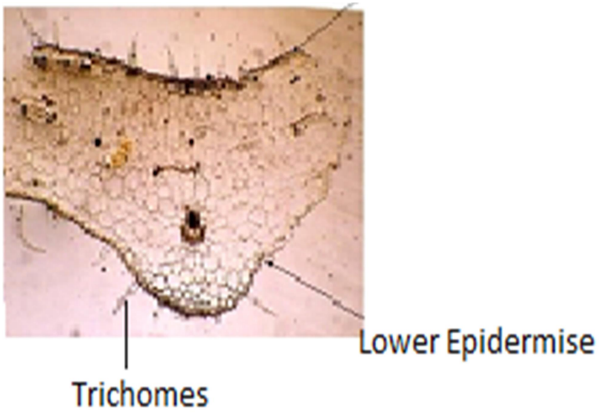

Figure 5: T.S of leaf

Table 2: Proximate values leaf of *Mimosa rubicaulis (Lam)*

Sr. No	Parameters	Values obtained w/won dry wt. basis
1	Moisture content	6.66%
2	Total Ash	7.35%
3	Water soluble ash	2.1%
4	Acid insoluble ash	5.5%
5	Water-soluble extractive	9.54%
6	Alcohol-soluble extractive	10.32%

Graph 1: Graphical representation of proximate values of leaf of *Mimosa rubicaulis (Lam)*

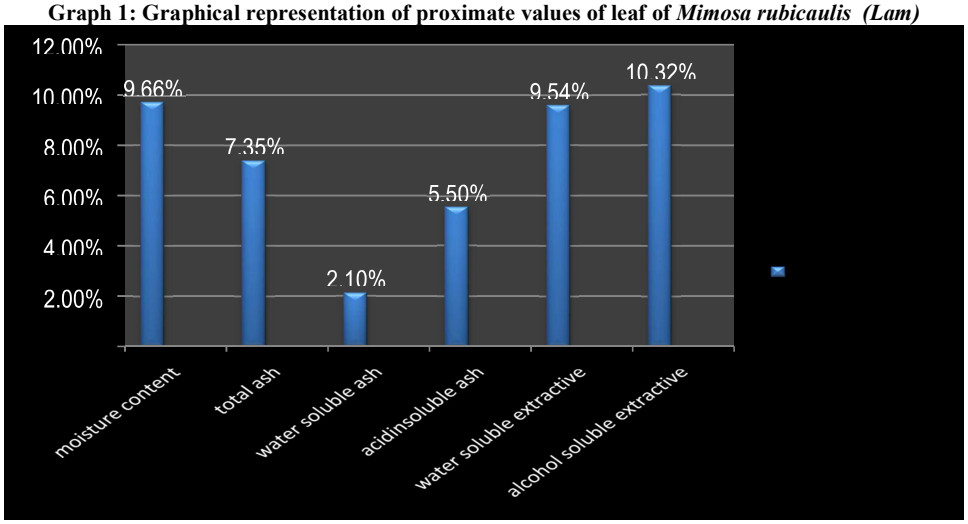

Table 3: Fluorescence evaluation of leaf *Mimosa rubicaulis* (*Lam.*)

Extracts of Different solvents	After 24 hours		After 48 hours	
	Normal light	UV light	Normal light	UV light
Petroleum ether	Brown	Brown	Dark brown	Dark brown
Chloroform	Green	Dark green	Green	Dark green
Ethyl acetate	Green	Green	Dark green	Dark green
Ethanol	Dark green	Dark green	Dark green	Dark green
Water	Dark green	Dark green	Dark green	Dark green

Table 4: The percentage yield by Soxhlet extraction method of crude extract of *Mimosa rubicaulis* (Lam)

Solvent	Weight of Powdered Plant Material	Volume of Solvent	Weight of Extract	% of yield of Extraction
Petroleum ether	500gms	200ml	9.8 gm	1.96
Chloroform			5.8 gm	1.16
Ethyl acetate			15.9 gm	3.18
Ethanol			25.6 gm	5.12
Aqueous			52.4 gm	10.48

Table 5: Qualitative Phytochemical Analysis of leaves extracts of *Mimosa rubicaulis* (Lam)

Chemical constituents	Tests	PEE	$CHCl_3E$	EAE	ETE	AQE
Alkaloids	Mayer s	+	–	+	–	+
	Wagner s	–	–	–	+	–
	Dragendroff's s	–	+	–	+	+
Flavonoids	Shinado	–	–	+	+	–
	Lead acetate	–	+	++	+++	++
	Alkaline reagents	+	–	+	++	+
Carbohydrates	Molisch's	–	–	+	+	–
	Barfoed's	+	+	–	–	+
	LibermannBurchard's	–	–	–	–	–
Phytosterols and Triterpenoids	Salkowski	+	+	–	+	+
	LibermannBurchard's	+	–	+	–	+
Glycosides	Legal's	–	+	+	+	+
	Borntrager's	–	–	+	–	–
	LibermannBurchard's	+	–	+	+	+
Saponins	Foam or Froth	–	+	+	+	+

(+++) Appreciable amount; (++) Moderate amount; (+) Trace amount; (-) completely absent Abbreviations: PEE: Pet-ether; CH_3ClE: Chloroform extract; EAE: Ethyl-acetate extract; ETE: Ethanol extract; AQE: Aqueous extract

Table 6: Quantitative Phytochemical Analysis of leaves extracts of *Mimosa rubicaulis* (Lam).

S. No	Phytochemicals	PEE(w/w)	$CHCl_3E$(w/w)	EAE(w/w)	ETE(w/w)	AQE(w/w)
1	Alkaloids	0.03	0.15	0.01	1.26	1.02
2	Flavonoids	0.05	0.25	2.32	3.33	2.29
3	Phenol	-	0.01	0.2	0.1	0.3
4	Saponin	0.02	0.1	0.11	0.13	0.1
5	Carbohydrate	0.03	0.15	0.01	1.26	1.02

DISCUSSION

Studies of physicochemical characterization can function a valuable source of data and are usually applied in judging the purity and quality of the drug. The extractive values indicate the chemical constitution of the drug. Within the present study, the extractive value of aqueous was the very best, followed by ethanol and then ethyl acetate. The ash value determines the poor matter or inorganic composition and other impurities present alongside the drug. The pharmacognostic standard for the leaves of *Mimosa rubicaulis* (Lam) is laid down for the primary time during this study. To conclude, this study might be used as a diagnostic tool for the standardization of this Herbal plant and can be helpful within the characterization of the crude drug. This study also concludes that leaves contain a number of pharmaceutically important phytochemicals like Alkaloids, Saponin, Flavonoids, Terpenoids, Tannins, Carbohydrates and Glycosides. An extra study of the extracts is ongoing to isolate, characterize, and elucidate the structure of the bioactive compounds present which were liable for potent pharmacological activity.

CONCLUSION

This is the first report on the pharmacognostic studies of *Mimosa rubicaulis* (Lam) and helpful in the description of the crude drug. Further phytochemical research is needed to identity the active product of *Mimosa rubicaulis* (Lam) may serve as leads in the development of new pharmaceuticals.The study of Microscopical or histological characteristics is useful in the detection of adulterants in both entire and powdered forms of crude drugs. Apart from variations in the cellular arrangement, many times, the type of cuticle of the epidermis and cell inclusion also helps in the detection of the adulterants. The percentage yield by the Soxhlet extraction method of crude extract of *Mimosa rubicaulis* is high in ethanol solvent. The present study showed that ethanolic and ethyl acetate extract of *Mimosa rubicaulis* (Lam) is rich in basic nutrients. Qualitative phytochemical screening showed that it is abundant in phytochemicals such as Alkaloids, Carbohydrates, Saponin, Flavonoid, Tannins, Terpenoids and Glycosides especially it was found in high amount of Flavonoid present in ethanolic and ethyl acetate extract than other extracts. Quantitative analysis showed that ethanolic extract contains higher amounts than ethyl acetate extracts. From the findings of the study, it may be concluded that the ethanolic extract of *Mimosa rubicaulis* (Lam) *acts* as the potential source of phytochemicals which may be used traditional medicine for the prevention of several diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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