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ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTI-ULCER ACTIVITY OF PHYTOCONSTITUENTS PRESENT IN *MORINGA OLEIFERA* LEAF

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ABSTRACT: *Moringa oleifera* leaf is widely used in the traditional medicine system and also used as a food by different communities and local tribes of Assam in India. *Moringa oleifera* leaf contains various bioactive compounds such as alkaloids, flavonoids, rutin, steroids, *etc.* The flavonoids quercetin act as an anti-ulcer agent, and rutin have a gastro cytoprotective effect *i.e.*, present in *M. oleifera* leaf. The main aims of the current study were to isolate and characterize the active compounds using column chromatography and spectroscopy, respectively, and evaluate the anti-ulcer activity of *Moringa oleifera* leaf extract. The FTIR analysis of isolated compounds indicated the presence of functional groups OH, C=C, C-Cl, C-Br. Besides these, the FTIR spectroscopy for ethanolic leaf extract confirmed the presence of COOH, O-H, N-H. The results of the IR analysis also reveal that the components of *Moringa oleifera* leaf could be aromatic or aliphatic alcohols or phenols, amine, ketones, esters, and some nitrogen-containing compounds. In *in-vitro* anti-ulcer screening, the ethanolic extract of *Moringa oleifera* possessed an antacid, antisecretory, anti-ulcer property which might be due to the presence of saponin. The current study results suggest that consumption of the leaves of *Moringa oleifera* may be beneficial in the healing of ulcers in patients suffering from peptic ulcer disease.

INTRODUCTION: Gastric acidity and ulceration are quite common, inflicting tremendous human suffering current days. Due to many factors, ulcers might produce in humans, such as stress, spicy fast food, chronic use of anti-inflammatory drugs, etc. [1]. Even though the etiology of ulcers is unknown in most cases, it is typically accepted that it is the result of unevenness between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism [2].

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The effective drug against peptic ulcers should either reduce the aggressive factors on gastroduodenal mucosa or increase mucosal resistance against them [3]. Despite established anti-ulcer drugs, a rational therapy for peptic ulcers remains elusive, and a search for safer potential drugs is being carried out. The development and progression of gastric ulcers depend on the type of food consumed by the patient.

It has been shown that spicy food, fatty food, or food containing caffeine stimulates acid secretion in the stomach [4] and high fiber diets such as potatoes, bananas, peas, beans, and so forth reduce duodenal ulcers' development. Previous reports on the incidence of gastric ulcers in the South Asian population reveal that the occurrence is lower due to the type of food consumed by this region; one of

the foods speculated to protect against ulcers is *Moringa oleifera* Lam. Leaves [5]. Furthermore, the flower bud of Moringa pterygosperma [6], a synonym of *Moringa oleifera* that is widely consumed in India, Pakistan, has been reported to possess anti-ulcer activity against aspirin-induced gastric ulcers in rats [7]. However, the effect of different extracts of leaves or fruits of *Moringa oleifera* on gastric and duodenal ulcers is not known. *Moringa oleifera* is a member of the Moringaceae family, which grows throughout the tropics.

It is native to sub-Himalayan tribes of northwest India, Pakistan, Bangladesh and Afghanistan, but now distributed worldwide in the tropics and found in many [8,9]. It was well known to the ancient world but recently has been "rediscovered" as a multipurpose tree with a tremendous variety of potential uses [10]. It is also an important food commodity that has enormous attention as the natural nutrition of the tropics. The leaves flower and immature pods from the moringa tree were used as a nutritive vegetable in many parts of the country [11, 12].

Many research papers reported that leaves of Moringa are highly rich in Beta carotene, protein, vitamin C, calcium, and potassium. It can also act as an effective source of natural antioxidant [13, 14, 15]. Apart from being high, nutritive Moringa can also be treated as an important medicinal plant for treating many prolonged diseases, including cancer [16].

Moringa provides the rare combination of zeatin, quercetin, sitosterol, caffeoylquinic acid and kaempferol. More than 40 natural anti-oxidants with numerous other secondary metabolites of health importance in Moringa species [17]. *Moringa oleifera* is well known for containing several bioactive compounds like flavonoids, triterpenes, steroids, alkaloids, *etc*. The flavonoid quercetin present in the leaves is well known anti-ulcer agent [18.] Further, the leaves contain rutin, a flavonoid that is reported to have a gastro cytoprotective effect [19].

The main aim was to isolate, characterize and identify phytochemicals from *M. oliefera* leaf concerning studying its anti-ulcer activity.

MATERIAL AND METHODS:

Collection and Identification of Plant Material: The leaves of *Moringa oleifera* were collected in the month of March 2019 from Nagaon, Assam. Identification of the plant species was done based on the plant's morphological and floral characters. The collected fresh leaves were identified by the Department of Botany, A.D.P. College, Nagaon, Assam.

Extraction of Dried Leaf Using Maceration Technique: The collected matured, and healthy leaves were thoroughly washed with water and dried under shade (room temperature). It was then pulverized to increase the surface area for extraction. 100 gm of the pulverized leaves of *Moringa oleifera* was soaked in n-hexane at room temperature for seven days. The crude extract obtained was decanted and filtered. The crude extract obtained was later concentrated in vacuo.

Isolation of Active Compounds from the Leaves of *Moringa oleifera*: In order to obtain pure isolates, the crude extracts were fractionated using Column Chromatography (CC).

Preparation of Column: A glass column of 18mm diameter (Borosil made) was used in this study. The column was packed with a wet packing method using silica gel for column chromatography and hexane solvent. Then the column was run with hexane before adding the sample to remove any impurities present inside the column. Then the column was run with a solvent system of Hexane and Dichloromethane (DCM) using gradient technique.

TABLE 1: COLUMN CHROMATOGRAPHY TIME PROFILE

S. no.	Day & Date	Mobile Phase	Mobile phase ratio	Eluent collected (ml)
1	Day 1	Hexane: DCM	10 : 90	100ml
2	Day 2	Hexane: DCM	20 : 80	100ml
3	Day 3	Hexane: DCM	30 : 70	100ml
4	Day 4	Hexane: DCM	40 : 60	100ml
5	Day 5	Hexane: DCM	50 : 50	100ml
6	Day 6	Hexane: DCM	60 : 40	100ml
7	Day 7	Hexane: DCM	70 : 30	100ml

Preparation of Mobile Phase: Hexane and Dichloromethane (DCM) were selected for the mobile phase, and the solvent system was introduced to the column in gradient technique. The solvent system was prepared of 100 ml using hexane and DCM. The mobile phase was prepared through solvent system fraction in different ratio- Hexane: Dichloromethane (10:90, 20:80, 30:70, 40:60, 50:50, 60:40,70:30, 80:20, 90:10).

Drying of Collected Column Eluents: The collected isolates were subjected to drying using vacuo to remove the solvent portion. When it was formed into dry crystal form, the following sample was processed for further characterization.

Characteristic of Isolated Compounds:

Determination of R_f Value Using TLC: Thin layer chromatography analysis of the crude extract of leaves of *Moringa oleifera* and isolates of the column were carried out. The solvent system used for development was DCM: diethyl ether: n-hexane in a ratio of 1:1:1. After development, the TLC plates were allowed to dry and viewed under the UV-lamp at 254 and 366 nm. The isolates were subjected to TLC to determine the R_f values.

Spectrometric Analysis (FTIR): The dried surface of the isolated compound and powder extract of MO were analyzed by Fourier Transform infrared spectroscopy (FTIR) (Shimadzu, IR Affinity 1, Japan). The infrared radiation is propagated through the sample to obtain the corresponding spectrum, which was averaged from several data acquisitions. The dried isolated compound and dried extract were encapsulated in 100mg of KBr pellet to prepare a translucent sample disc. FTIR spectra were acquired in the wavenumber range of 400-4000 cm^{-1}. After each measurement, the crystalline surface was washed with demineralized water and dried with a soft paper.

***In-vitro* Antiulcer Screening of Ethanolic Leaf Extract of *Moringa oleifera*:**

Preparation of Ethanol Extract of *Moringa oleifera* Leaf: The freshly collected leaves of *Moringa oleifera* were first washed with distilled water and dried. The dry leaves were then powdered (100 gm) and macerated with petroleum ether to remove fatty substances and marc was further exhaustively extracted with 90% ethanol for 3 days. Then the crude extract obtained was decanted and filtered. Later the crude extract obtained was dried in vacuo to remove the solvent portion. The prepared extract was subjected to TLC.

Pepsin Induced Artificial Stomach Acid Assay (Acid Neutralizing Capacity): The anti-ulcer activity was determined by evaluating the neutralization of gastric juice through Pepsin induced artificial stomach acid assay. For the preparation of artificial gastric juice, 2 gm of NaCl and 3.2 gm of pepsin were dissolved in 500 ml distilled water. Hydrochloric acid (7.0 ml) and adequate water were added to make a 1000 ml solution. The pH of the prepared solution was adjusted to 1.2. Here the efficacy of the extract in regards to neutralizing the gastric juice was determined by measuring the pH with digital pH meter [20, 21].

RESULTS:

Percentage (%) Yield of N-Hexane Extract of *Moringa oleifera* Leaf:

TABLE 2: PERCENTAGE (%) YIELD OF *MORINGA OLEIFERA* LEAF

S. no.	Plant materials(gm)	Percentage (%) yield
1	100gm	6.41

Physical and Characteristic Study of Isolates Collected Through Column Chromatography:

TABLE 3: CHARACTERISTICS OF ISOLATES

S. no.	Fractions/Eluents	Appearance	Quantity (ml)
1	Fraction A, B, C	Orange	100ml+100 ml+ 100ml
2	Fraction D	Light yellow	100ml

Determination of R_f Value of Crude Extract & Isolates of Column:

TABLE 4: RF VALUE OF CRUDE EXTRACT

S. no.	Sample	Rf (cm)
1	Ethanolic leaf Extract	0.9

TABLE 5: RF VALUE OF ISOLATES OF COLUMN

S. no.	Samples	Rf (cm)
1	Fraction A	0.90
2	Fraction B	0.90
3	Fraction C	0.90
4	Fraction D	0.98

On the basis of the similar R_f value and other physical and preliminary characteristics of each fraction and available literature reports it was noticed that the Fraction A, Fraction B, and Fraction C eluent of Column were identical in nature, thus combined together and named as "Fraction ABC". Then the "Fraction ABC" was processed for spectroscopic analysis

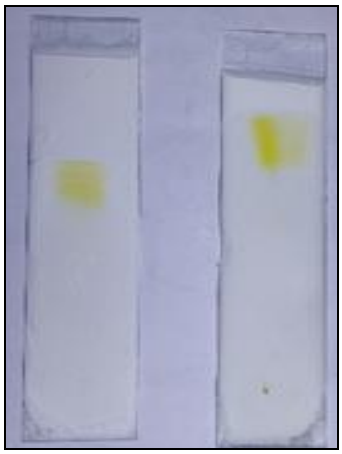

FIG. 1: TLC OF ISOLATED COMPOUND

FIG. 2: TLC OF CRUDE EXTRACT

FTIR Analyses of the Isolate (Fraction ABC was the Isolate from Column Chromatography) and Moringa Extract Powder:

Spectroscopic Analysis of Isolated Compound I.E. "Fraction ABC:

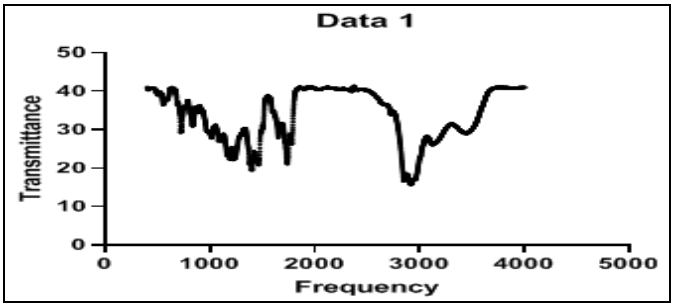

FIG. 3: FTIR SPECTRA OF ISOLATED COMPOUND ABC (FRACTION ABC)

TABLE 6: FUNCTIONAL GROUPS WITH THEIR WAVE NUMBER FOUND IN FTIR SPECTRA OF FRACTION ABC

S. no.	Wavenumber (cm^{-1})	Functional group
1	2100, 2182	C=C(alkene)
2	3355, 3500	O-H(alcohol)
3	2730	P-H
4	3031	=CH
5	638	C-Cl
6	564	C-Br

Spectroscopic Analysis of Crude Extract of *Moringa oleifera*:

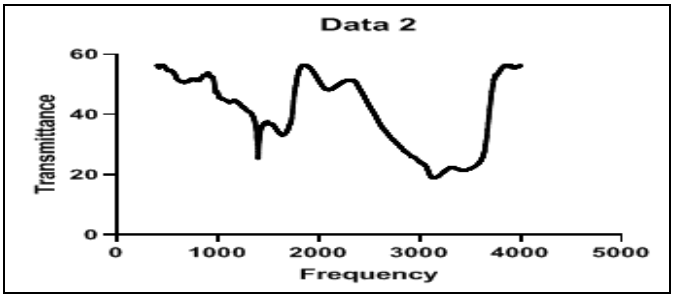

FIG. 4: FTIR SPECTRA OF CRUDE ETHANOLIC EXTRACT OF MORINGA OLEIFERA

TABLE 7: FUNCTIONAL GROUPS WITH THEIR WAVE NUMBER FOUND IN FTIR SPECTRA OF ETHANOLIC EXTRACT OF *MORINGA OLEIFERA*

S. no.	Frequency range	Functional group
	2598, 2649	-COOH
	3300	O-H
	3377, 3420	N-H

In-vitro* Antiulcer Screening of Ethanolic Extract of *Moringa Oleifera:

TABLE 8: PH OF THE ARTIFICIAL GASTRIC JUICES AT TIME INTERVAL

S. no	Quantity of gastric juice	pH after 5 mins	pH after 10 mins
1	10ml	1.36	1.57
2	10ml	1.29	1.39
3	10ml	1.13	1.25

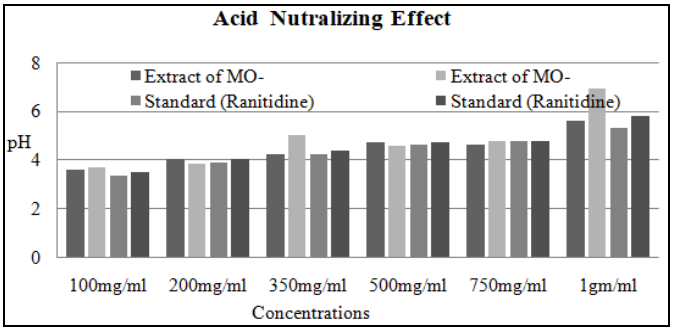

FIG. 5: pH OF THE OF GASTRIC JUICES AFTER ADDING DIFFERENT CONC. OF EXTRACTS AND STANDARD (RANITIDINE)

TABLE 9: pH OF THE OF GASTRIC JUICES AFTER ADDING DIFFERENT CONC. OF EXTRACTS AND STANDARD (RANITIDINE)

S. no.	Concentration	Extract of *Moringa oleifera*		Standard (Ranitidine)	
		pH after 5 Min	pH after 10 Minu	pH after 5 Min	pH after 10 Min
1	100mg/ml	3.56	3.7	3.33	3.5
2	200mg/ml	4.00	3.85	3.9	4.00
3	350mg/ml	4.22	5.00	4.20	4.35
4	500mg/ml	4.72	4.55	4.60	4.70
5	750mg/ml	4.60	4.78	4.75	4.74
6	1gm/ml	5.6	6.9	5.3	5.8

DISCUSSION: *Moringa oleifera* has an impressive range of medicinal uses with high nutritional value. This study is similar to the study of Ogundele *et al.* [22], in our study 100 gm of *Moringa oleifera* leaf was preceded for hexane/DCM extraction process. Later, column chromatography was performed in gradient technique by the selected solvent system hexane/DCM. As a result of four types of fraction components *viz* Fraction A, Fraction B, Fraction C, Fraction D collected as eluent. However, after performing TLC of these eluents, we got the same Rf value (0.9cm) of Fraction A, Fraction B, Fraction C; and 0.98cm R_f value of Fraction D. Although Fraction A, Fraction B, Fraction C appeared as orange colour and Fraction D as a light yellow colour.

On the basis of the similar R_f value and other similar physical and preliminary characteristics of each fraction and available literature reports, the Fraction A, Fraction B, and Fraction C eluent of Column were found to be identical in nature; thus, these were combined together and named as "Fraction ABC". The colour of the dried Fraction ABC was clear Orange and crystalline in nature. The FTIR analysis of isolated compound indicated the presence of OH group at 3355,3500 cm^{-1} stretching vibrations; C=C band at 2182 and 2100 cm^{-1} stretching vibration; P_H at 2730 cm^{-1}; C-Cl at 633 cm^{-1};C-Br at 546 cm^{-1}. Besides these, we performed FTIR spectroscopy for ethanolic leaf extract of *Moringa oleifera* also, and it resulted in COOH at 2598 and 2649 cm^{-1} stretching vibration; O-H stretch at 3300 cm^{-1}; and N-H at 3377, 3420 cm^{-1} stretching vibration. The results of the IR analysis also reveal that the components of *Moringa oleifera* leaf could be aliphatic or aromatic. It may, therefore, be inferred that aromatic or aliphatic alcohols or phenols, amine, ketones, esters, and some nitrogen-containing compounds are some of the constituents of the leaf of *Moringa oleifera* [23, 24]. However, OH function and N-H stretch suggest that aromatic or aliphatic phenols or alcohols and nitrogen-containing molecules are major components of the *Moringa oleifera* leaf. One study performed by V. Sharma *et al.*, also reported the presence of similar functional groups and R_f values for the components of *Moringa oleifera* [25]. From both the study it can be suggested that the isolated compound is saponin in nature. Further spectroscopic analysis may confirm and identify the saponin. In this work, we have also analyzed the infrared absorption from leaves of *Moringa oleifera* as an alternative technique to investigate the main functional groups present.

The prepared ethanolic extract of *Moringa oleifera* leaf was analyzed for TLC. The R_f value of the extract was found to be 0.9. Based on folklore claims and traditional uses, the prepared extract was screened to evaluate its anti-ulcer property through *in-vitro* (Pepsin induced artificial gastric juice) model. The pH of the prepared artificial gastric juice was measured and was found to be 1.2. Here the efficacy of the extract in regards to neutralizing the gastric juice was determined by measuring the pH at a time interval. The different extract concentrations showed the time-based result concerning neutralizing the artificial gastric juice. **Fig. 5** illustrated the time-based results of acid neutralization by ethanolic extract and standard drug. The ethanolic extract of MO showed the maximum acid-neutralizing effect at the concentration of 1gm/ml by elevating the pH of the artificial gastric juice from 1.2 to 6.9. On the contrary standard drug (ranitidine) elevated the pH of the artificial gastric juice from 1.2 to 5.8. On analysis of results obtained against all the concentrations, it was noticed that the extract of MO was most effective at conc. of 1 gm/ml and least effective in the conc. of 100 mg/ml in respect

to neutralizing the artificial gastric juice. The data reported here indicate that the ethanolic extract of Moringa oleifera may possess an antacid, antisecretory, anti-ulcer property, which may be due to the presence of saponin other compounds in the mixture [26, 27]. The current study results suggest that consumption of the leaves of *Moringa oleifera* may be beneficial in the healing of ulcers in patients suffering from peptic ulcer disease.

CONCLUSION: In this research work, a crystalline substance was isolated from n-hexane extract of pulverized Moringa leaves. The exact structure of this compound is yet to be ascertained. The spectral data available suggest aliphatic alcohol. The ethanolic extract of *M. oleifera* showed a promising acid-neutralizing effect. We can conclude that the plant screened herein showed promising pharmaceutical utility in light of these findings.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest. All the tables and figures are self-made and original.

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