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EVALUATION OF ANTIOXIDANT AND ANTACID ACTIVITIES OF *GLOBIMETULA BRAUNII*, *MICRODESMIS PUBERULA* AND *PARQUETINA NIGRESCENS* EXTRACTS

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ABSTRACT

Natural products are one of the best sources of therapeutic-based compounds for the treatment of peptic ulcer, which denotes open sores on the lining of the stomach or small intestines. *Globimetula braunii* (Family Loranthaceae), *Microdesmis puberula* (Family Pandaceae) and *Parquetina nigrescens* (Family Apocynaceae) have been documented for various medicinal uses, including the traditional treatment of peptic ulcer in Nigeria. This study aimed to assess the antioxidant and antacid properties of *Globimetula braunii*, *Microdesmis puberula* and *Parquetina nigrescens*. The plant samples were collected, identified and authenticated. The samples were allowed to air dry, pulverised and then extracted using methanol. The extracts were assessed for antioxidant (2,2-diphenyl-1-picrylhydrazyl, DPPH), free radical scavenging activity, total phenolic content (TPC), total flavonoid content (TFC), and antacid activity. The total phenolic content was highest in *G. braunii*, followed by *P. nigresens* and then *M. puberula* with values of 6.74 mg GAE/g, 3.35 mg GAE/g and 0.56 mg GAE/g, respectively. The crude extract of *G. braunii* showed the highest radical scavenging activity below 50% with IC_{50} value of 53.73. *Globimetula braunii* methanol extract (250 mg/250mL) showed the highest artificial gastric acid neutralising activity by raising the pH of the artificial gastric acid from 1.2 to 1.80±0.04, which is like the standard $NaHCO_3$ (100 mg/250mL) that raised the pH of the artificial gastric acid from 1.2 to 1.78±0.03. The results from this study support the ethnomedicinal claims that plants can be used in the management of peptic ulcer, and these plants could serve as sources of lead compounds to produce natural antacids.

INTRODUCTION

There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of these, peptic ulcer is the most common among people. It is erosion of lining of stomach or the duodenum [1]. Peptic ulcer disease (PUD) is characterised by discontinuation in the gastrointestinal (GI) tract's inner lining due to gastric acid secretion or pepsin. It expands into the stomach epithelium's muscularis propria layer. While it typically occurs in the stomach and proximal duodenum, the jejunum, distal duodenum or lower esophagus may also be affected. Patients with gastric ulcer typically have epigastric pain 15–30 minutes after eating, whereas those with duodenal ulcer typically experience pain 2-3 hours after eating [4].

Peptic ulcer disease are mainly caused by *Helicobacter pylori* (*H. pylori*), NSAID's (non-steroidal anti-inflammatory drugs), increased gastric acid secretions, and other factors like stress, smoking, spicy food, and nutritional deficiencies. PUD includes gastric ulcers, with a lifetime prevalence of 5 to 10% of patients. This number is probably low because some patients may not have any symptoms. Research has indicated that the incidence of stomach ulcer rises with advancing age and the duration of NSAID use [2]. Prostaglandins, mucus, growth factors and sufficient blood supply protect the gastric mucosa against damage. However, the gastric mucosa can be damaged by alcohol, smoking, hydrochloric acid, ischemia, NSAIDs, hypoxia, and *Helicobacter pylori infection* [3].

The antacids act by neutralising the acid in the stomach and by inhibiting pepsin, which is a proteolytic enzyme. Each of these cationic salts has a characteristic pharmacological property that determines its clinical use. Antacids have therapeutic uses such as heartburn symptoms in GERD, duodenal and gastric ulcers, stress gastritis, pancreatic insufficiency, non-ulcer dyspepsia, diarrhea caused by bile-acid, biliary reflux, constipation, osteoporosis, urinary alkalinisation, phosphate binding in chronic renal failure [5].

Antioxidants protect the living system from oxidative insults, which is a hallmark feature of cancer, cardiovascular disease, diabetes and many other diseases [6]. The prevalent synthetic anti-ulcer drugs available for the management and treatment are effective. However, their adverse side effects which include, abdominal pain, constipation, diarrhea, nausea, and vomiting pose a major limitation to the deliberate use of these drugs.

Numerous scientific disciplines acknowledge the important and valuable role that medicinal plants play, including the food industry, the fragrance and cosmetics sector, and other medical and pharmaceutical procedures [7, 8]. Some medicinal plants have been previously reported to treat gastric ulcers in Nigeria [9-12]. Antacids heal ulcers through the elimination of gastric acid by neutralisation. However, they do not decrease the volume of gastric secretions. Different antacid drugs vary markedly in their *in vivo* and *in vitro* potency, and that should be considered when antacids are prescribed.

Globimetula braunii (Engl.) Danser, a parasitic plant belonging to the Loranthaceae Family, is indigenous to the central tropical region of Africa. The plant is widely distributed in tropical African countries such as Ghana, Cameroun, and Nigeria and is a major ingredient used in herbal medicines [13]. *G. braunii* is known to be beneficial in Nigeria for treating wound infections, cholera, diabetes, hypertension, cancer, and gastrointestinal tract ailments [14, 15].

Microdesmis puberula Hook.f. ex Planch. (Family Pandaceae) is commonly known as the fruity branch. It grows in tropical Africa; Nigeria to the Central African Republic and Uganda,

south to D.R Congo. In Nigeria, a leaf decoction is taken to treat acute spleen pain. The stem of *M. puberula* is used to treat erectile dysfunction. Stress-associated conditions like pain could also be treated with *M. puberula* [16, 17].

Parquetina nigrescens (Afzel.) Bullock, belonging to the family Apocynaceae, is commonly known as African Parquetina. It is a shrub native to tropical West Africa that has long been used in traditional medicine. The leaves, roots, and latex of the plant are parts that are traditionally utilised in medicine. The plant is known by the Yoruba name "Ewe Ogbo," which translates to "The leaf that hears," and is widely utilised in traditional medicine practices in the western region of Nigeria. Additionally, it is believed to be effective in curing almost any illness, as seen by its use in incantations in the western region of Nigeria [18].

The biological activities reported in the literature for *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens* are mostly carried out on different morphological parts. *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens* are traditionally used for the treatment of peptic ulcers in Nigeria. The study therefore aimed to evaluate the antioxidant and antacid activities of *Globimetula braunii*, *Parquetina nigrescens* and *Microdesmis puberula*.

RESULTS

Phytochemical screening

The dry leaf samples of *G. braunii, M. puberula* and *P. nigrescens* were screened for alkaloids, flavonoids, tannins, anthraquinones, and cardiac glycosides. The result showed the presence of alkaloids, saponins, flavonoids and tannins in the three samples. Cardiac glycoside was absent in *P. nigrescens* and anthraquinone was absent in all the plant samples.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The Total phenolic content (TPC) was determined by the Folin-ciocalteu method, using the Gallic acid equivalent/g of the extract by reference to the standard. The total flavonoid contents (TFC) are reported as mg quercetin equivalent/g of extract by reference to the standard curve. The TPC was highest in *G. braunii*, followed by *P. nigresens* and then *M. puberula* with values of 6.74 mg GAE/g, 3.35 mg GAE/g and 0.56 mg GAE/g, respectively. The TFC was highest in *G. braunii*, followed by *M. puberula* and then *P. nigrescens* of values 1.99 mg QE/g, 1.36 mg QE/g and 0.79 mg QE/g, respectively (Table 1).

Table 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the crude methanol extract *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens*

Extract	TFC (mgQE/g)	TPC (mgGAE/g)
Globimetula braunii	1.99±0.04	6.74±0.15
Microdesmis puberula	1.36±0.12	0.56±0.18
Parquentinanigrescens	0.79±0.25	3.35±0.59

DPPH Free Radical Scavenging Assay (FRSA)DPPH Radical scavenging capacity. The scavenging activity is expressed as a percentage of the ratio of the decrease in absorbance of the test solution to that of the DPPH solution without the plant extracts. It was observed that the free radical scavenging activity of the plant extracts was dose-dependent, with 200 µg/mL dose having the highest activity for all extracts, while 6.25 µg/mL had the lowest across all the samples tested, so with an increase in dose, an increase in activity was observed. The crude extracts of *P. nigrescens, M. puberula* and *G. braunii*) showed radical scavenging activity below 50% with IC_{50} values 528.126µg/mL, 130.894 µg/mL and 53.735 µg/mL, respectively (Figure 1).

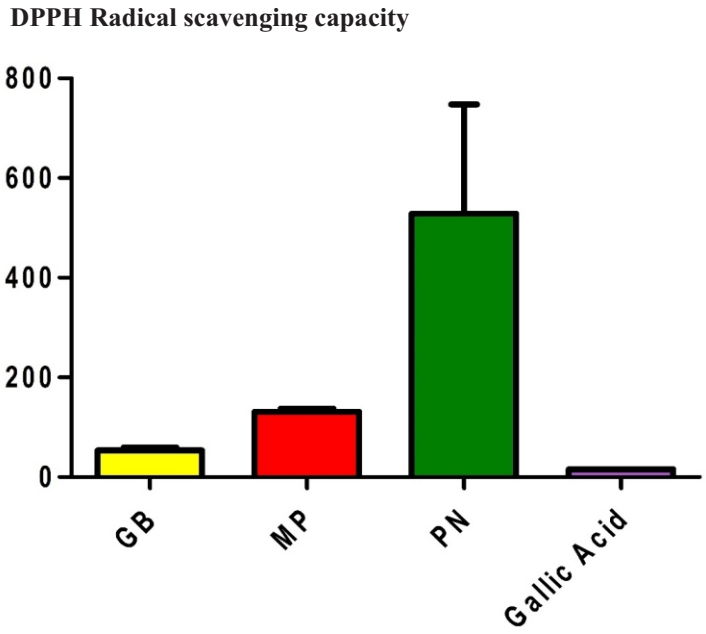

Figure 1: IC_{50} value of the DPPH Free Radical Scavenging Activity of the crude methanol extract of *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens*

Key:
GB - *Globimetula braunii*
MP- *Microdesmis puberula*
PN- *Parquetina nigrescens*

Artificial Gastric Acid Neutralising Activity

The artificial gastric acid-neutralising activity of *G. braunii, M. puberula and P. nigrescens* extracts is shown in Table 2 .*G. braunii* methanol extract (250 mg/250mL) showed the highest artificial gastric acid neutralising activity by raising the pH of the artificial gastric acid from 1.2 to 1.80±0.04, which is similar to the standard $NaHCO_3$ (100 mg/250mL) that raised the pH of the artificial gastric acid from 1.2 to 1.78±0.03 and *M. puberula* methanol extract had the lowest artificial gastric acid neutralising activity raising the pH from 1.2 to 1.67±0.03, which was higher than the negative control (distilled water) that raised the pH of artificial gastric acid from 1.2 to 1.30±0.00 (Table 2). The amount of artificial gastric juice consumed to pH 3 (Action efficiency) showed that *G. braunii* extracts consumed 1.30±0.10 mL of artificial gastric juice, which is lesser than the standard, sodium bicarbonate which consumed 13.85 ±0.35 mL of artificial gastric juice (Table 2).

Table 2: *In vitro* antacid activity of the crude methanol extracts *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens*

Extract/ Standard	Concentration (mg/250mL)	pH	Neutralising efficiency	Action efficiency (Amount of AGT consumed in mL)	Number of H+ ion consumed
Sodium carbonate	250	8.94	1.53±0.11	13.85±0.35	0.87±0.02
	100	8.72	1.78±0.03	6.60±1.00	0.42±0.06
GB	250	7.51	1.80±0.04*	1.30±0.10****	0.08±0.01****
	100	8.02	1.68±0.04*	0.90±0.10****	0.06±0.01****
MP	250	7.05	1.67±0.03*	0.80±0.10****	0.05±0.01****
	100	7.61	1.82±0.04**	0.65±0.15****	0.04±0.01****
PN	250	7.09	1.720±0.04*	0.80±0.10****	0.05±0.00****
	100	7.86	1.740±0.01*	0.65±0.05****	0.04±0.00****
***Water**	100	6.19	1.30±0.00		

Key: GB – *Globimetula braunii;* **PN** – *Parquetina nigrescens;* **MP** – *Microdesmis puberula*

DISCUSSION

It was observed in this study, that the crude extracts of *G. braunii, M. puberula*and *P. nigrescens* contain phenolics and flavonoids. The TPC and TFC of*G. Braunii* were higher than the other two plants. There was no difference in the TFC values reported by Akpanyunget al. [22], for the ethanol root extract of *M. puberula*. Phenolics and flavonoids are important constituents of fruits, vegetables and beverages. These compounds show a wide range of antioxidant activities *in vitro* and are thought to exert protective effects against major diseases such as cancer and cardiovascular diseases [23].

The intensity of the radical scavenging effect is measured by calculating half-inhibition concentration (Ic_{50}), which is the efficient concentration required for decreasing initial free radicals' concentration by 50%. The radical scavenging activity (DPPH) of the crude extracts *G. braunii, M. puberula* and *P. nigrescens* was compared to the standard (Gallic acid) as shown in Figure1. The antioxidant effect of the aqueous, methanol and flavonoid extracts of leaves of *Parquetina nigrescens* were found to increase in a concentration-dependent manner and possessed more antioxidant activity according to a report by Ayoola et al. [24]. Phenolic compounds influence antioxidant activity measurements, it interferes with the oxidation process by reacting with free radicals (ROS), chelate pro-oxidant metal ions, scavenge oxygen, and prevent oxidative degradation of lipids [25]. A previous report of antioxidant activity of *G. braunii* ethanol extract gave the IC_{50} value of 31.21 µM, compared to 53.73 μgmL^{-} in the present study [26]. Acheampong et al. [27] also evaluated the antioxidant activity of *M. puberula*. The IC_{50} of methanol and petroleum ether extract and the reference drug (Ascorbic acid) with regard to the DPPH scavenging activity were 1.1 μgmL^{-}, 1.2 μgmL^{-}, and 0.2 μgmL^{-} respectively. Plants' *in vitro* antioxidant activity can be impacted by genetic, environmental, and physiological variables that alter the composition and concentrations of these molecules [28]. The differences in antioxidant activity and flavonoid concentration are largely determined by ecogeographic parameters, specifically longitude, and altitude [29].

The antacid activity of *G. braunii, M. puberula* and *P. nigrescens* crude extract was evaluated using the artificial gastric acid neutralising activity. Hyperacidity is a common gastrointestinal disorder which may not necessarily be brought about by a pathogenic infection. It is attributed to a functional disorder that can result due to a variety of reasons, which is related to heartburn and gas gastric juices from the stomach into the lower oesophagus [30].

Antacids are essential to prevent and treat gastrointestinal (GI) issues. These medications are frequently used to lessen the symptoms of illnesses related to acid reflux and to decrease the production of stomach acid. Antacids can lower the risk of gastritis, peptic ulcer disease (PUD), and gastroesophageal reflux disease (GERD) by preventing mucosal damage brought on by excessive gastric acid production. Antacids aid in the repair of mucosal surfaces by reducing stomach acid production and easing symptoms like regurgitation, dyspepsia, and heartburn. Antacids offer symptomatic relief by decreasing acid reflux and easing related symptoms such as heartburn, chest pain, and regurgitation [31]. Antacids include Aluminum hydroxide, magnesium hydroxide, or magnesium trisilicate [32].

Antioxidants are compounds that considerably impede or delay the oxidation of an oxidizable substrate, even at concentrations much lower than that of the substrate. Free radical and hydroxyl free radical scavengers have the physiological function of guarding against cellular component damage caused by chemical processes involving free radicals.

Free radicals can be neutralised or stabilised by antioxidants prior to their attack on biological targets and cells. As a result, they are essential for preserving the best possible cellular and systemic health and wellbeing [33].

G. braunii, M. puberula and *P. nigrescens* possess minimal antioxidant properties which could be due to the minimal flavonoid and phenolic components present in them. The plants possess high antacid activity; this could denote that other

constituents in synergism with the medium flavonoid and phenolic compounds could be responsible for the antacid activity shown by these three plants [34].

The results from this study showed that *G. braunii, M. puberula* and *P. nigrescens* possess artificial gastric acid neutralising activity, which implies that they can be used as antacids to reduce the acidity of the stomach. As a result, the plants' capacity to raise the pH of the stomach and hence lessen its acidity was used to assess their suitability for treating ulcers. This is the first report on the antacid potential of *G. braunii, M. puberula* and *P. nigrescens* in literature.

Antacids comprise a major over-the-counter class of drugs sold worldwide. Patients seeking treatment for heartburn and acid reflux disease spend billions of dollars on these over-the-counter remedies. Antacids relieve the symptoms of GERD, acid reflux disease (GERD), heartburn, hyperacidity, and upset stomach [35]. Antacids work by inhibiting the proteolytic enzyme pepsin and neutralising excess hydrochloric acid (HCl) in gastric juice [36].

Although the three plant species have been reportedly used to manage ulcers ethnobotanically, no extensive research has been done to evaluate their antacid prowess. However, there are other antiulcer reports on all three plantspecies. Odetolaet al. [37] reported the gastrointestinal protective property of aqueous leaf extract of *P. nigrescens* in rats by influencing the growth and development of gastric mucosa and reducing basal acid secretion. Okany et al. [17] reported the antiulcerogenic activity of methanol stem extracts of *M. puberula*, by decreasing the stress-induced stomach ulceration and increasing ulcer index, while the antiulcer activity of methanol root or stem extract of *G. braunii* has been reported by Oluwole et al. [38]. The results of the present study showed that all the methanol crude extracts of *G. braunii, M. puberula* and *P. nigrescens* had artificial gastric acid neutralising activity in that they were able to raise the pH of artificial gastric acid from 1.2 to 1.80±0.04 for *G. braunii*, 1.67±0.04 for *M. puberula*, 1.72±0.04 for *P. nigrescens* when compared to that of the standard $NaHCO_3$ (1.53±0.11). Our findings showed that *G. braunii, M. puberula* and *P. nigrescens* extracts possessed artificial gastric ulcer-neutralising activity, which implies that they can be used in the management of gastriculcers.

CONCLUSION

Notable phytochemicals included alkaloids, flavonoids, tannins, and saponins. To the best of our knowledge, no research has been done to assess the selected plant species' antacid capabilities. Variations in the phytochemical principles and activities were noted, and they could be related to the harvesting process, the kind of soil at the harvest area and the surrounding environmental factors. The plant species' antacid and antioxidant properties encouraged their use as an antacid for relief of ulcer symptoms. The study's findings validate the traditional medical beliefs that *G. braunii*, *M. puberula* and *P. nigrescens* can be used to relief ulcer symptoms. Further study on these plants may also provide lead chemicals for the development of natural antiulcer agents.

MATERIALS AND METHODS

Plant collection

The leaves of *G. braunii*, *M. puberula* and *P. nigrescens* were collected from Ajibode, Ibadan, Oyo State Nigeria in November 2020. The plants were identified and authenticated at Forest Herbarium Ibadan (FHI) by Mr. A. J. Egunjobi where voucher specimens were deposited with voucher numbers FHI 113310 (*Globimetula braunii*), FHI 113311 (*Mirodesmispuberula*) and FHI 113169 (*Parquetina nigrescens)*. Every authenticated plant sample was placed in duplicate at the Department of Pharmacognosy Herbarium University of Ibadan (DPHUI).

Preliminary phytochemical screening was performed on the plants' powdered samples using standard methods [39, 40] to screen for the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones, and cardiac glycosides.

Plant extraction

The leaves were air-dried for two weeks at room temperature in the shade. After that, they were crisp-dried for ten minutes at 40°C in a regulated oven before being ground into powder. Two hundred grams of ground-up samples for each of the three samples were extracted by maceration in 2.5 L of 100% methanol for 72 hours. A glass rod was used to periodically stir the liquid to improve extraction. Whatman's No. 1 filter paper was then used for filtering. A rotary evaporator (Buchi, Rotavapour R-210, Switzerland) was used to evaporate the filtrate *in vacuo*, and a water bath set at 40°C was used for the final solvent removal and drying. The crude extracts were stored in sterile air-tight bottles for bioassay and further analysis. The percentage yield of the extracts was calculated using the formula below:

$$\% \text{ Yield of crude extract} = \frac{W.E}{W.S} \times 100$$

W.E = weight of extract; W.S = weight of powdered sample

Measurement of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the crude extracts *Globimetula braunii, Microdesmis puberula* and *Parquetina nigrescens* were evaluated following the method of Khatoon et al. [41], with slight modifications. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g. Folin-Ciocalteu 2.5 mL diluted 10 folds was added into 0.5 mL aliquot of each crude extract at 200 µg/mL and was allowed to stand for 3 min after which 2 mL of (75 g/L) Na_2CO_3 solution in distilled water was added to the mixture. The standard gallic acid was prepared in seven concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL). The experiment was prepared in triplicates. Content of the mixture was thoroughly mixed and incubated at room temperature for 30 min. The blank was set up with 0.5 mL methanol, 2.5 mL of Folin-Ciocalteu and 2 mL of (75 g/L) Na_2CO_3. Absorbance of mixture was read at 765 nm after 30 minutes of incubation using UV-VIS spectrophotometer (Spectrumlab 752S). A linear dose response regression curve was generated using absorbance reading of gallic acid concentrations (200-3.125 µg/mL) at wavelength of 765 nm. The total phenolic content in the plant extract was calculated using the formula:

$$TPC = \frac{CV}{M}$$

Where TPC is the Total phenolic contents in mg GAE/g of dry weight of extracts, C is the concentration of equivalent gallic acid established from calibration curve µg/mL, V is the volume of extract (5 mL) and M is the weight of plant extract (0.03 g).

Measurement of Total Flavonoid Contents (TFC)

The aluminium chloride colorimetric method used in this study

was according to Ebrahimzadeh et al. [42], with slight modifications. Quercetin was used as a standard for the calibration curve. Three mg of quercetin was dissolved in methanol and then diluted in the range of 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL, the diluted standard solution (0.5 mL) was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. Three milligrams of the extracts (*G. braunii, M. puberula* and *P. nigrescens*) were dissolved in 20 mL of methanol, and then 0.5 mL of each extract together with the reagents used for the standard was mixed thoroughly in a test tube. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in the blank. Incubation was done at room temperature for 30 min and the absorbance of the reaction was measured at 415 nm using a UV-VIS spectrophotometer (Spectrumlab 752S). A yellow colour indicated the presence of flavonoids. The experiment was carried out three times. Based on the measured absorbance, the concentration of flavonoids was read from the calibration curve. The content of the flavonoid in extract was expressed in terms of quercetin (mg quercetin/g of extract).

The Total Flavonoid Contents in the plant extract was calculated using the formula below:

$$TFC = \frac{CV}{M}$$

Where, TFC is the Total Flavonoid Contents mg QE/g of dry weight of extracts, C is the concentration of equivalent quercetin established from calibration curve µg/mL, V is the volume of extract (5 mL) and M is the weight of plant extract (0.03 g).

DPPH Radical Scavenging Activity

The free radical scavenging activity of the crude extracts *G. braunii, M. puberula* and *P. nigrescens* was evaluated according to the method described by Adeniran and Sonibare [43], with slight modifications. The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability with gallic acid as positive control. One (1) mL of methanol solution of test samples and standard (gallic acid) at different concentrations (200, 100, 50, 25, 12.5, 6.25 µg/mL) were mixed separately with 3 mL (0.004%) of freshly prepared 2,2- diphenyl-1-picryl-hydrazyl-hydrate (DPPH-Sigma Aldrich). For the blank, 1 mL of methanol was mixed with 3 mL of DPPH. The reaction mixtures were incubated at room temperature and allowed to react for 30 minutes in the dark. After 30 min, the absorbance was measured at 517 nm using UV-VIS spectrophotometer (Spectrumlab 752S) and converted into a percentage of antioxidant activity. The results were recorded in triplicate. The degree of decolorisation of DPPH from purple to yellow indicates the scavenging efficiency of the extract. The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from a calibration curve using the equation of the graph.

The percentage of inhibition of DPPH (%) was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of each sample was expressed in terms of IC50

Artificial Gastric Acid Neutralising Activity

The artificial gastric acid neutralising assay was carried out as described by Sandhya et al.[44].

Preparation of Extracts

Crude extracts (250 mg and 100 mg) of *G. braunii, M. puberula* and *P. nigrescens* were weighed separately and dissolved in 5 mL of methanol and then made up to 250 mL with distilled water.

Preparation of Artificial Gastric Juice

Two grams of salt and 3.2 mg of pepsin enzymes were dissolved in 500 mL water. Then 7.0 mL hydrochloric acid and adequate water were added to make a 1000 mL solution of the artificial gastric acid at pH 1.20.

Determination of pH of the Prepared Extracts

Out of the prepared extracts 90 mL was collected and used for the pH determination at temperatures ranging from 25– 37 ºC. The pH values of the control solution were also determined according to the method described by Wu et al. [45].

Determination of the neutralising effects on artificial gastric acids

Test solutions of 90 mL each were added to 100 mL of artificial gastric juices at pH 1.2. The pH values were determined to examine the neutralising effect [8, 9, 40].

***In vitro* titration method of Fordtran's model for determination of the neutralisation capacity**

About 90 mL of test samples were placed in a 250 mL beaker and warmed to 37 °C and aeration was given at 136 air bubbles per minute to imitate the peristaltic movements by using a magnetic stirrer. The test samples were titrated with the artificial gastric juice to obtain an endpoint of pH 3.The consumed volume (V) of artificial gastric juice was noted. The total consumed hydrogen ion (milli-Mole) was measured as 0.06309 (m Mol) × V (mL) [8, 9, 40].

Statistical Analysis

For the statistical computations, Graph Pad Prism Version 4.00 for Windows (Graph Pad Software Inc.) was employed. The collected experimental data were reported as mean ± SEM, where SEM stands for standard error of the mean. One-way analysis of variance (ANOVA) was used to compare the groups, with the Dunnett Multiple Comparisons Test taking into account Test Vs control. The differences were statistically significant when $P<0.01$ where $n=6$.

Abbreviations

GB	*Globimetula braunii*
MP	*Microdesmis puberula*
PN	*Parquetina nigrescens*
DPPH	2, 2- diphenyl-1-picrylhydrazyl
ANOVA	Analysis of variance

Availability of data and materials

Data and materials are available upon request.

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