

Full Length Research Paper

Antibacterial and antioxidant activities of some selected plants used for the treatment of cattle wounds in the Eastern Cape

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This study was carried out to determine the antimicrobial and antioxidant properties of selected medicinal plants (*Aloe ferox* Mill, *Ptaeroxylon obliquum* (Thunb) Radlk. and *Calpurnia aurea* (Aiton) Benth) used for the treatment of cattle wounds. DPPH 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt (ABTS), radical scavenging assays were used to determine the antioxidant activity of selected plants. Microbial susceptibility assay using the minimum inhibitory concentration (MIC) was used to test plant extracts against gram-negative and gram-positive bacterial strains. Each extract was also assayed to determine the total phenol, flavonoid, flavonol and prothocyanidin contents. The methanol extracts produced better results in all the assays. All plant extracts inhibited both gram-positive and gram-negative bacteria but there was more inhibition on gram-positive strains. Plant extracts tested also showed considerable scavenging activities against both DPPH and ABTS radicals at 0.2 mg/ml. These results are probably due to the level of phenols, flavonoids and flavonols in the extracts. The antibacterial and antioxidant activities displayed by these plants might justify their traditional use in wound healing and out of the three species tested, *P. obliquum* displayed the best antibacterial and antioxidant properties.

Key words: Antimicrobial, cattle, flavonoid, medicinal plants, phenol, scavenging activity, wound healing.

INTRODUCTION

One of the major threats to optimal cattle production is wounding, which results from various causes such as castration, dehorning, tail docking, ear tagging, wire cuts, tick bites, bush thorns among others (AHA, 2007). Wounds are physical injuries that result into an opening or break of the skin and have been defined by livestock farmers in South Africa to include sores, abscesses, warts and inflamed skin lesions (Luseba et al., 2007). Open wounds are particularly prone to infection, especially by bacteria and also provide an entry point for myiasis-causing flies. In addition, the wound healing

process may be hampered by the presence of oxygen free radicals that cause the destruction of cells and tissues (Houghton et al., 2007).

In South Africa, the use of herbal remedies for the treatment of livestock diseases is widely practiced by the small-scale farmers especially in the rural communities (Masika et al., 2000). Although, rural farming communities now have access to commercial medicines, many still prefer and rely on cheaper traditional remedies rather than the relatively expensive commercial ones. Medicinal plants have therefore, become the focus of intense study in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Jäger et al., 1996).

A survey of the traditional remedies use by rural farmers in the treatment of cattle wounds and myiasis in the

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Eastern Cape Province of South Africa revealed a variety of plants (Soyelu and Masika, 2009). Three plant species (*Aloe ferox* Mill. *Ptaeroxylon obliquum* (Thunb) Radlk and *Calpurnia aurea* (Aiton) Benth) were selected based on predominant use and availability at the time of the study. These plants were screened for antibacterial and antioxidant activities in an endeavour to confirm the rationale behind their acclaimed traditional use.

MATERIALS AND METHODS

Collection and preparation of plant materials

The leaves of *A. ferox*, *P. obliquum* and *C. aurea* were collected from Amatola Basin (32°41'S and 26°59'E) in the Eastern Cape Province, South Africa. Voucher specimens were deposited at the Giffen herbarium of the University of Fort Hare. Fresh leaves (100 g) of each plant were macerated separately and extracted with 1000 ml of distilled water and methanol by shaking on an orbital shaker for 24 h. The mixture was filtered first by using muslin cloth and then Whatman's No. 1 Filter paper after which, the water extract was freeze-dried while the methanol extract was concentrated to dryness under reduced pressure at 40°C. Each extract was stored in a glass bottle and later re-dissolve in their respective solvents to the desired concentrations for the various experiments.

Chemicals

All the chemicals and solvents used were of analytical grade. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), butylated hydroxytoluene (BHT), 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt (ABTS), gallic acid, catechin, potassium ferricyanide, rutin, quercetin and FeCl₃ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); sodium carbonate and Folin-Ciocalteu reagent were from Merck Chemical Supplies (Darmstadt, Germany) while vanillin was from BDH Chemicals Ltd. (Poole, England).

Bioassay

Eight bacteria species comprising of four gram-positive (*Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10702, *Bacillus pumilus* ATCC 14884) and four gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 19582, *Klebsiella pneumoniae* ATCC 10031 and *Enterobacter cloacae* ATCC 13047), were selected to assess susceptibility patterns against the extracts prepared in the present study. Each organism was maintained on nutrient agar slant (Biolab) and later recovered for testing by growth in nutrient broth no. 2 (Biolab) for 24 h.

Plant extracts (0.1, 0.5, 1.0 and 5.0 mg/ml) in nutrient agar medium was poured into Petri dishes (90 mm), swirled and left overnight for solvent to evaporate. Thereafter, each bacteria culture was diluted 1:100 with fresh sterile nutrient broth and streaked in radial patterns on the agar plates (Afolayan and Meyer, 1997). Plates were incubated at 37°C and examined after 24 h. Two blank plates containing only nutrient agar and two containing nutrient agar and methanol without the extracts were served as controls. Tetracycline was used as the standard control and each test was replicated three times.

Determination of total phenols

The total phenolic contents in the plant extracts were determined

according to the modified folin-ciocalteu procedure (Wolfe et al., 2003). 1 ml of each extract (1 mg/ml) was mixed with 5 ml folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The mixtures were vortexed for 15 s and allowed to stand for 30 min at 40°C. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VS spectrophotometer and the total phenolic content was expressed as mg/g gallic acid equivalent using the equation based on the calibration curve: $y = 8.098x + 0.187$. R² = 0.92, where x is the absorbance and y was the gallic acid equivalent (mg/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordoñez et al. (2006). 0.5 ml of 2% aluminum trichloride ethanol solution was added to 0.5 ml of each extract. After one hour at room temperature, the absorbance was measured at 420 nm. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve:

$$y = 43.86x - 0.175, R^2 = 0.993$$

Where, x is the absorbance and y is the quercetin equivalent (mg/g).

Determination of total proanthocyanidins

The procedure reported by Sun et al. (1998) was used to determine the total proanthocyanidin in the extracts. A volume of 0.5 ml of 1 mg/ml of extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min and the absorbance was measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the equation based on the calibration curve:

$$y = 1.638x - 0.004, R^2 = 0.992$$

Where, x is the absorbance and y is the catechin equivalent (mg/g).

Determination of total flavonol

Total flavonol content was determined by using the method of Yermakov et al. (1987). 2 ml (20 g/l) of aluminum trichloride and 3 ml (50 g/l) sodium acetate solutions was added to 2 ml of the extract (1 mg/ml). The absorbance at 440 nm was read after 2.5 h at 20°C. Flavonol content was expressed as rutin equivalence (mg/g) using the following equation based on the calibration curve:

$$y = 5.467x - 0.004, R^2 = 0.992$$

where, x is the absorbance and y is the rutin equivalent (mg/g).

Radical scavenging assays

DPPH radical scavenging assay

The effect of plant extracts on DPPH radical was estimated using the method described by Liyana-Pathirana and Shahidi, (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of varying concentrations (0.025 – 0.4 mg/ml) of extracts in methanol. The reaction mixture vortexed thoroughly was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using

Table 1. Antibacterial activity of the methanol extracts of *A. ferox* (AF), *P. obliquum* (PO) and *C. aurea* (CA) leaves.

Bacterial species	Gram+/-	Minimum inhibitory concentration (mg/ml)			
		AF	PO	CA	Tetra ^a
<i>S. aureus</i>	+	5.0	1.0	5.0	0.025
<i>S. faecalis</i>	+	na	5.0	5.0	0.008
<i>B. cereus</i>	+	na	0.5	na	0.006
<i>B. pumilus</i>	+	na	1.0	na	0.012
<i>E. coli</i>	-	5.0	5.0	5.0	0.001
<i>P. aeruginosa</i>	-	na	na	na	0.002
<i>E. cloacae</i>	-	na	na	na	0.001
<i>K. pneumoniae</i>	-	na	0.5	na	0.005

na, Not active; ^a, Tetracycline (standard antibiotic).

BHT as a standard reference and a solution of DPPH (in methanol) mixed with methanol (solvent) served as the negative control. This assay was replicated three times and the radical scavenging activity calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample/std}}) / (\text{Abs}_{\text{control}})] \times 100}{}$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical + methanol; $\text{Abs}_{\text{sample/std}}$, the absorbance of DPPH radical + sample extract/standard. The actual decrease in absorption induced by the test compounds was compared with the positive controls.

ABTS radical scavenging assay

The method of Re et al. (1999) was adopted for the ABTS radical scavenging assay. The stock solution, which was allowed to stand in the dark for 16 h at room temperature, contained equal volume of 7 mM ABTS salt and 2.4 mM potassium persulfate. The resultant ABTS^+ solution was diluted with methanol until an absorbance of about 0.70 ± 0.01 at 734 nm was reached. Varying concentrations of the plant extracts (1 ml) were reacted with 1 ml of the ABTS^+ solution and the absorbance taken at 734 nm between 1 and 3 min using the spectrophotometer. The percentage inhibition was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample/std}}) / (\text{Abs}_{\text{control}})] \times 100}{}$$

Where, $\text{Abs}_{\text{control}}$ was the absorbance of ABTS radical + methanol; $\text{Abs}_{\text{sample/std}}$ the absorbance of ABTS radical + sample extract / standard.

Statistical analysis

Experimental results were expressed as mean \pm standard deviation (SD) of three replicates and sample means were separated using Duncan's Multiple Range tests (SAS, 1999).

RESULTS

Antibacterial activity

The methanol extract of all the three plants inhibited both gram-positive and gram-negative bacteria but there was

more inhibition on gram-positive strains (Table 1). No inhibition however, was observed from the water extracts of all the plants tested. Methanol extracts of *A. ferox* and *C. aurea* inhibited the growth of *S. aureus* at 5.0 g/ml and *P. obliquum* at 1.0 mg/ml. Of all the plants tested at 5.0 mg/ml, the highest concentration used, also inhibited the growth of *E. coli*; only *P. obliquum* was observed to have been able to inhibit the growth of *K. pneumoniae* whereas none of the plant extracts was effective against *P. aeruginosa* and *E. cloacae* at the concentrations tested.

Total phenolic, flavonoid, flavonol and proanthocyanidin contents

Generally, the methanol extracts of the three plants possessed higher phenolic, flavonoid, flavonol and proanthocyanidin contents than their aqueous counterparts. However, an exception to this result is the water extract of *A. ferox*, which contained higher prothocyanidin content than its methanol extract. Both the methanol and water extracts of *P. obliquum* possessed the highest phenolic content (Table 2). It is also of interest to note that, the methanol extract of *C. aurea*, which exhibited the highest level of flavonoids, flavonol and proanthocyanidin contents possessed lower phenolic content.

DPPH radical scavenging activity

Figure 1 shows the DPPH radical scavenging activity of the methanol and water extracts of *A. ferox*, *P. obliquum* and *C. aurea* when compared with the standard (BHT) at varying concentrations. It was observed that, the methanol extracts of *P. obliquum* and *A. ferox* showed high radical scavenging activity at 0.2 mg/ml when compared with other extracts. At this concentration, the scavenging effects were 92.3 and 79.2%, respectively. The water extract of *P. obliquum* also showed a good scavenging effect of 69.9% at the same concentration. However, the scavenging effect of the methanol extract of *C. aurea* was

Table 2. Total phenol, flavonoid, flavonol and proanthocyanidin contents of the leaf extracts of *A. ferox*, *P. obliquum* and *C. aurea* (n = 3, x ± S.D).

Extracts	Total phenol	Flavonoids	Flavonol	Proanthocyanidins
Methanol				
<i>A. ferox</i>	81.58 ± 15.62 ^c	19.53 ± 2.60 ^c	19.57 ± 3.68 ^c	5.70 ± 2.21 ^c
<i>P. obliquum</i>	155.79 ± 6.62 ^a	29.17 ± 0.12 ^b	208.50 ± 24.53 ^b	28.98 ± 8.28 ^b
<i>C. aurea</i>	76.87 ± 3.86 ^c	142.50 ± 11.34 ^a	299.01 ± 4.05 ^a	49.82 ± 11.28 ^a
Water				
<i>A. ferox</i>	49.86 ± 2.05 ^d	13.79 ± 1.45 ^c	13.78 ± 1.85 ^c	11.52 ± 3.84 ^c
<i>P. obliquum</i>	105.66 ± 5.40 ^b	15.96 ± 0.48 ^c	24.43 ± 6.11 ^c	16.08 ± 4.74 ^c
<i>C. aurea</i>	47.48 ± 3.66 ^d	18.97 ± 1.07 ^c	19.84 ± 0.13 ^c	14.88 ± 4.16 ^c

Values in the same column with a common superscript are not significantly different at P ≤ 0.05.

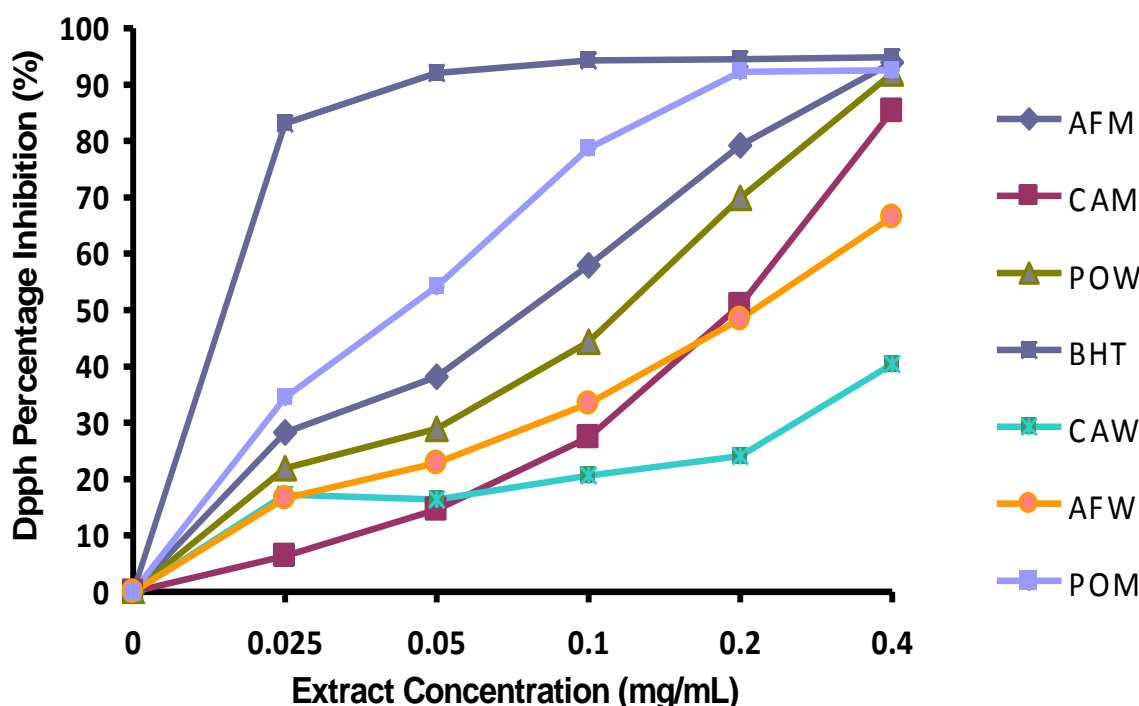


Figure 1. DPPH radical scavenging activities of the water and methanol extracts of *A. ferox*, *P. obliquum* and *C. aurea*.

Where, AFM, *A. ferox* methanol extract; CAM, *C. aurea* methanol extract; POW, *P. obliquum* water extract; BHT, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt; CAW, *C. aurea* water extract; AFW, *A. ferox* water extract; POM, *P. obliquum* methanol extract. (Each value is expressed as mean ± SD (n = 3)).

only moderate (50.8%) at 0.2 mg/ml.

ABTS radical scavenging activity

The methanol extract of *P. obliquum* was a fast and effective scavenger of ABTS radical (Figure 2) comparable to the standard (BHT). All the extracts however, effectively scavenged the ABTS radical with inhibition percentage ranging from 90.3 to 102.9% at a concentration of 0.2 mg/ml.

DISCUSSION

Antibacterial activity

Gram-negative bacteria especially *E. coli* are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market (Afolayan, 2003). Therefore, the ability of the methanol extracts of *A. ferox*, *P. obliquum* and *C. aurea* to inhibit *E. coli* is noteworthy even though it was at the highest concentration (5.0 mg/ml) used. A number of micro-

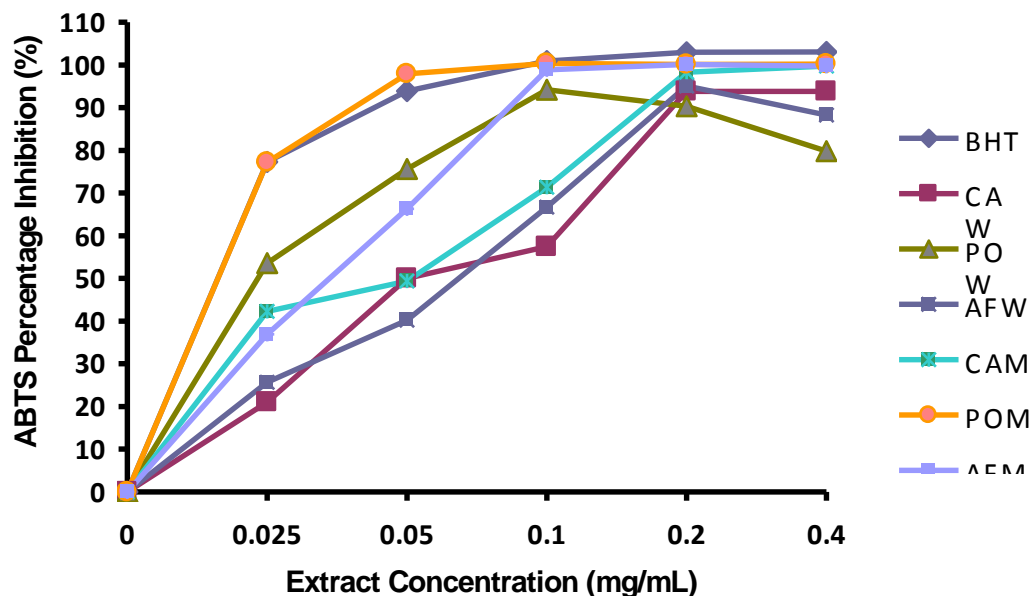


Figure 2. ABTS radical scavenging activities of the water and methanol extracts of *A. ferox*, *P. obliquum* and *C. aurea*.

Where, BHT, 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt; CAW, *C. aurea* water extract; POW, *P. obliquum* water extract; AFW, *A. ferox* water extract; CAM, *C. aurea* methanol extract; POM, *P. obliquum* methanol extract; AFM, *A. ferox* methanol extract (each value is expressed as mean \pm SD (n = 3)).

organisms have been found to infect wounds, among which are *S. aureus*, *P. aeruginosa*, *E. coli*, *S. faecalis*, *Clostridium perfringens* and *enterococcus* (Bowler et al., 2001). According to Odimegwu et al. (2008), the inhibition of microbial contaminants of wounds allows the normal tissue repair process to occur.

The water extracts of the plants were tested because traditionally, plant extracts are prepared with water but the results were not surprising because other researchers have reported that, their antibacterial activities are low or most of the time not detectable (Matu and van Staden, 2003; Luseba et al., 2007). This is probably because remedies used by the farmers are administered in large quantities and in high concentrations. Hence, they are still able to meet the required physiological levels in animals. Therefore, the antibacterial activity showed by the methanol extracts of *A. ferox*, *P. obliquum* and *C. aurea* might justify their traditional use in wound healing.

Total phenolic, flavonoids, flavonol and proanthocyanidin contents

The methanol extracts were generally more active than the water extracts as antioxidant agents. This could possibly be due to a higher concentration of tannins in the methanol extracts as compared to the water extracts (Frum and Viljoen, 2006). Both extracts of *P. obliquum* (methanol and water) with the highest level of phenols

also exhibited the greatest antioxidant activity. Phenolic compounds are rich in hydroxyl groups and are believed to have the ability of free radical scavenging and antioxidation. The activity of polyphenols, which are the major plant compounds with antioxidant activity, is believed to be mainly due to their redox properties (Zheng and Wang, 2001; Rosidah et al., 2008). Flavonoids are phenolic compounds, which are also very effective antioxidants. However, extracts with higher phenolic content do not always have higher flavonoid content (Maisuthisakul et al., 2007). This was evident for the extracts of *C. aurea*, which had a higher total flavonoid and flavonol content when compared with that of *P. obliquum* and *A. ferox*, even though the total phenolic content was lower. According to this study, different plant extracts contain different levels of total flavonoids as a proportion of the total phenolic compounds. The presence of these phytochemicals in good quantity in the three plants, therefore explain their good radical scavenging activity.

Radical scavenging activities

The DPPH method detects free radical scavengers and uses a reduction in intensity of the coloured free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Its use is advantageous in evaluating antioxidant effectiveness because it is more stable than the hydroxyl and super-oxide radicals

(Siriwardhana and Shadidi, 2002; Hough-ton et al., 2005). The methanol extract of *C. aurea* showed moderate antioxidant activity when compared with an early study by Adedapo et al., (2009), where at a concentration of 0.1 mg/ml, the DPPH and ABTS scavenging effects of the methanol leaf extracts reached 89.7 and 100%, respectively. This could be due to differences in geographical coordinates, time of collection of plant materials as well as the method of extraction (Celiktas et al., 2007). However, the three plants studied showed considerable scavenging activity, which is an indication that they could serve as free radical inhibitors.

The scavenging ability of the extracts was found to be higher in the ABTS radical scavenging assay than the DPPH and this may be explained by the difference in the mechanisms involved in the radical-antioxidant reactions (Yu et al., 2002). Although, some compounds that have ABTS scavenging activity do not show DPPH scavenging activity (Wang et al., 1998), all the plant extracts tested in this study showed good and significant scavenging active-ties against both DPPH and ABTS radicals. Compounds with free radical scavenging activity have been shown to prevent oxidative damage to tissues and significantly improve wound healing (Svobodová et al., 2006).

The overall results of the antioxidant and antibacterial assays in this study suggest the rationale behind the traditional use of *A. ferox*, *P. obliquum* and *C. aurea* for the treatment of cattle wounds. Out of the three species, *P. obliquum* displayed the best antibacterial and antioxidant properties. The obtained results could therefore form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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