



Phytochemical, Antioxidant and Antibacterial Screening of the Leaves of *Barleria dinteri* (Oberm), *Grewia flava* (DC) and *Jatropha lagarinthoides* (Sond)

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ABSTRACT

The study entails the screening of the leaves of three medicinal plants; *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides*, for presence of phytochemicals, as well as possession of antioxidant and antibacterial properties. The leaf samples of the three medicinal plants were collected from Zebediela in Limpopo province, South Africa. The extracts of the dried leaf samples were screened for the presence of phytochemicals using standard chemical tests, as well as the possession of antioxidant and antibacterial properties using DPPH assay and modified bioautographic procedure, respectively. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins and tannins in all medicinal plants under study, whereas anthraquinones and reducing sugars were only present in *Grewia flava* and *Jatropha lagarinthoides*. The leaves of all three plants showed possession of free radical scavenging activity against DPPH (an indication of antioxidant properties), as well as growth inhibition against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The current study indicates the presence of important phytochemicals, antioxidant and antibacterial properties in the leaves of *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides* which support the plants usage in African traditional medicine.

Keywords: Medicinal plants; Screening; Phytochemicals; Antioxidant; Antibacterial

INTRODUCTION

For a long time, people of the world continue to rely on medicinal plants as solutions to health problems [1]. In South Africa, over sixty percent of the rural black population still relies on medicinal plants infusions (herbal teas) and concoctions administered by traditional healers for their health problems in preference to/or concurrently with conventional synthetic drugs [2]. This rural reliance on medicinal plants is encouraged by several factors including: the lower cost of medicinal herbs compared to conventional synthetic drugs, efficacy and accessibility of herbal remedies, as well as their associated lower side effects compared with synthetic drugs [3]. South Africa is the habitat of a wide range of medicinal plants, distributed across its length and breadth, with many of these plants being used by local people for medicinal purposes [4]

Three medicinal plants, *Barleria dinteri* (Oberm), *G. flava* (DC) and *J. lagarinthoides* (Sond), are among the widely used in traditional medicine for the treatment of various diseases in the Limpopo province. *Barleria dinteri* commonly known as 'Leather leaf- barleria' (English) belongs to the family of Acanthaceae, is a perennial shrub that grows to a height of about 1.35 meters and is widely distributed in Southern Africa [5]. The leaves are used in traditional medicine to promote the healing of wounds, to treat some intestinal tumours, infectious diseases and to relieve joint pains and toothache [6]. *Grewia flava*, common name 'Raisin bush' (English), belongs to family of Tiliaceae and is widely distributed in the Southern African region throughout the North West province, Northern Cape and Limpopo (South Africa); the South Western areas of Zimbabwe, Namibia, Botswana and as far north as Zambia [2]. The leaves of *G. flava* are used in traditional medicine to treat stomach complications emanating from bacterial infections, particularly in children. *Jatropha lagarinthoides* belongs to the Euphorbiaceae family and is mainly found in Magaliesberg and Eastern parts of South Africa, growing in well-drained soil. The leaves of *J. lagarinthoides* are used in traditional medicine for the treatment of stomach complications, especially when food poisoning is suspected [7]. However, little is reported on the constituent active ingredients and biological activities of these medicinal plants. The current

study deals with the screening of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* from Limpopo province (South Africa) for presence of specific phytochemicals, as well as possession of antioxidant and antibacterial properties.

EXPERIMENTAL SECTION

Plant material

The leaf samples of the three medicinal plants; *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides*, were collected from Bolahlakgomo village in the Zebediela sub-district of the Limpopo province, South Africa. The identity of the plants was authenticated by Dr Bronwyn Egan, a botanical taxonomist at the department of Botany, University of Limpopo, and voucher specimen were deposited into the Herbarium.

Experimental procedure

Phytochemical screening

The dried ground leaves of the three medicinal plants were screened for the presence of alkaloids, anthraquinones, flavonoids, reducing sugars, saponins and tannins using standard procedures for the qualitative detection of these phytochemicals [8-13] as follows:

Test for alkaloids: About 50 g of dried leaf powders of selected medicinal plants species were extracted with about 50 ml of methanol on a boiling water bath for 20 min. The resultant extracts were then filtered, allowed to cool and dispensed in 2 ml aliquots into different test tubes. Wagner's alkaloidal reagent [2 g of iodine and 6 g of potassium iodide in 100 ml of distilled water (dH₂O)] was then added to each tube. Formation of reddish brown coloured precipitates indicated the presence of alkaloids.

Test for anthraquinones (Borntrager's test): Chloroform extracts of each of the three medicinal plants were obtained by warming the plant material-chloroform mixture over the water bath. Then 1 ml of dilute ammonia solution (10 %) was added to 2 ml of the extract and the mixture shaken. Formation of the reddish colour on the ammoniacal layer showed the presence of anthraquinones.

Test for flavonoids: Aqueous leaf extracts of the three medicinal plants were dried up on a boiling water bath. The resultant residues were then treated with sodium hydroxide (0.1 M), followed by addition of hydrochloric acid (0.1 M). The appearance of a yellow colour on addition of sodium hydroxide that turned colourless on addition of hydrochloric acid indicated the presence of flavonoids.

Test for reducing sugars (Fehling's test): Two millilitres of the water extract of ground leaves of each of the three medicinal plants was transferred into a test tube, to which 1 ml each of Fehling's solutions A (6.93 g of copper sulphate in 100 ml dH₂O) and B (0.44 g of sodium potassium tartrate and 13.0 g of sodium hydroxide) were added. The mixture was then shaken and heated in a water bath for 10 min. The appearance of a red precipitates indicated the presence of reducing sugars.

Test for saponins: About 2 g of the dried ground leaf samples of the selected medicinal plants were boiled in about 20 ml of distilled water over a water bath and filtered. The resultant extracts were then transferred into separate test tubes, shaken vigorously and then left to stand for 10 min. Formation of a persistent froth indicated the presence of saponins.

Test for tannins: About 0.5 g of the dried powdered leaf samples of plant species under study was boiled in 20 ml of water and then filtered into test tubes. The filtrates were then treated with few drops of 15 % ferric chloride solution. Formation of a dark green colour or blue colour indicated the presence of tannins.

Antioxidant activity screening

The dried ground leaves of the three medicinal plants were each extracted with n-hexane, dichloromethane, acetone and methanol using serial exhaustive extraction (SEE) procedure. The leaf extracts were then screened for antioxidant activity using 2, 2-diphenyl-1-picrylhydrazil (DPPH) (Sigma-Aldrich, USA) assay, as described by Masoko and Eloff [14]. Briefly, 10 µl of the extracts (10 mg/ml) were loaded onto the baseline of the TLC plates (Silica gel 60 F₂₅₄, Merck) and compounds were separated with three mobile phases, *viz*: benzene: ethanol: ammonia hydroxide, BEA (90:10:1, v/v/v), non-polar/basic; chloroform: ethyl acetate: formic acid, CEF (5:4:1, v/v/v), intermediate polarity/acidic and ethyl acetate: methanol: water, EMW (40:5.4:5, v/v/v), polar/neutral [15]. The developed TLC plates were dried and stained with 0.2 % DPPH in methanol to visualize specific bands that possess free radical scavenging activity. The plates were observed for 5 min after spraying with DPPH, with DPPH discolouration to yellow indicating the presence of antioxidant activity.

Antibacterial activity screening

The dried ground leaves of the three medicinal plants were each extracted with n-hexane, dichloromethane, acetone and methanol using serial exhaustive extraction (SEE) procedure. The leaf extracts were then screened for antibacterial activity by significant modification of the bioautographic procedure reported by Begue and Kline [16] and adapted for antifungal studies by Masoko and Eloff [17]. The n-hexane, dichloromethane and acetone leaf extracts of the medicinal plants were each re-dissolved in acetone and loaded onto TLC plates (Silica gel 60 F₂₅₄, Merck) and left to dry. TLC plates were sprayed with actively growing bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) without development in mobile phases and incubated in a humidified environment. After 24 h, the TLC plates were sprayed with 0.2 mg/ml of *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, USA) to visualize antibacterial activity. Activity was indicated by clear (non-coloured) zones on the TLC plates.

RESULTS

The dried ground leaves of the three medicinal plants were screened for the presence of alkaloids, anthraquinones, flavonoids, reducing sugars, saponins and tannins, and the results are shown in Table 1. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins and tannins in all medicinal plants under study, while anthraquinones and reducing sugars were only present in *Grewia flava* and *Jatropha lagarinthoides*.

Table 1: Qualitative phytochemical composition of the leaves of the three medicinal plants

Phytochemical group	<i>B. dinteri</i>	<i>Grewia flava</i>	<i>J. lagarinthoides</i>
Anthraquinones	-	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Reducing sugars	-	+	+

+: indicates presence; -: indicates absence

The leaf extracts of the three medicinal plants obtained with n-hexane, dichloromethane, acetone and methanol extracts were screened for antioxidant activity using TLC-DPPH assay and the results are shown in Figures 1a, 1b and 1c. The leaf extracts of all three medicinal plants showed the presence of compounds with antioxidant activities that were separated with mobile phases of different polarities by TLC. Impressive antioxidant activity was seen with the extracts obtained by relatively polar solvents, acetone and methanol.

The three leaf extracts of the medicinal plants; namely n-hexane, dichloromethane and acetone were screened for antibacterial activity using modified bioautographic procedure and the results are shown in Figure 2. The acetone extracts of all medicinal plants showed impressive growth inhibition against all test organisms, whereas the dichloromethane extracts of all medicinal plants showed moderate activity. The n-hexane extract of *G. flava* also showed impressive growth inhibition against all test organisms.

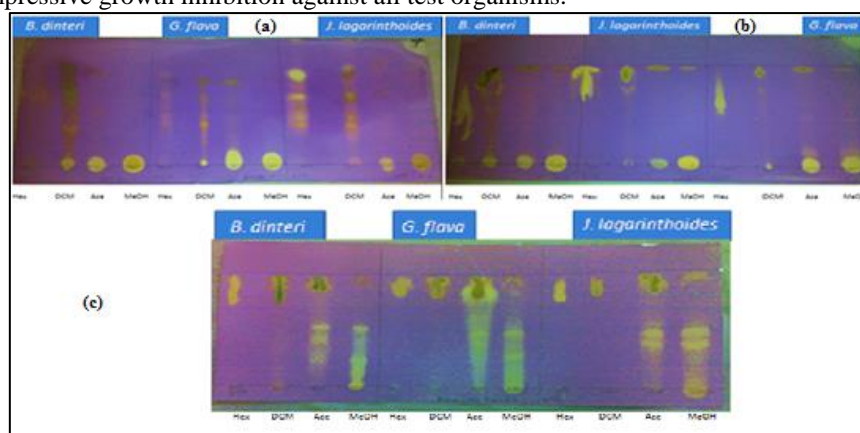


Figure 1: (a): Free radical scavenging activity of the leaf extracts (10 mg/ml) of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH. The chromatogram developed using BEA (90:10:1 v/v/v) mobile phase; (b): Free radical scavenging activity of the leaf extracts (10 mg/ml) of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH. The chromatogram developed using CEF (5:4:1 v/v/v) mobile phase; (c): Free radical scavenging activity of the leaf extracts (10 mg/ml) of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH. The chromatogram developed using EMW (40:5.4:5 v/v/v) mobile phase

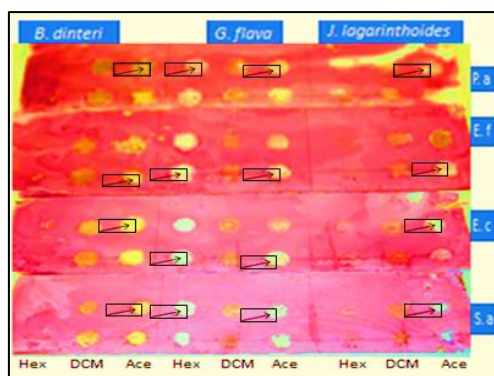


Figure 2: Bioautographic determination of the antibacterial activity of the leaf extracts (10 mg/ml) of *B. dinteri*, *G. flava* and *J. lagarinthoides*. P. a: *P. aeruginosa*, E. f: *E. faecalis*, E.c: *E. coli* and S.a: *S. aureus* (bacterial growth inhibition is highlighted by enclosed arrows on the figure)

DISCUSSION

Screening of plants extracts for the presence of phytochemicals is an important step in the studies of medicinal plants. In this study, the leaves of three medicinal plants were found to possess groups of phytochemicals that include alkaloids, anthraquinones, flavonoids, reducing sugars, saponins and tannins. Phytochemicals are reported to have various biological activities that contribute to the medicinal properties of medicinal plants [8-12]. The results of this study showed the presence of compounds with antioxidant activity within the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides*, as evidenced by reduction of purple DPPH to yellow colour that is indicative of free radical scavenging capability [14]. The antioxidant activities of the leaf extracts of the three medicinal plants were graded based on the intensity of the resultant DPPH bleaching yellowish colour, with the more colour intensity demonstrating impressive activity. Impressive activity was seen with the acetone and methanol extracts for all plants (Figures 1a, 1b and 1c.), which were shown by the intensity of the yellow colour resultant from the discolouration of purple DPPH. Compounds with free radical scavenging activity in the leaf extracts of the three medicinal plants under study were better resolved with the relatively more polar mobile phase (EMW) than with the intermediate (CEF) and the nonpolar (BEA) mobile phases. Unlike the compounds in the hexane leaf extracts of *B. dinteri* and *G. flava*, compounds with free radical scavenging activity in the hexane extract of *J. lagarinthoides* were also resolved with the nonpolar mobile phase, BEA, indicating diversity in the polarity of antioxidant compounds.

The results of this study also indicate the presence of components that have antibacterial activity against test organism within the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides*, as evidenced by the cleared (non-coloured) spots on the TLC plates against reddish metabolised INT solution [16,17]. The antibacterial activities of the leaf extracts of the three medicinal plants were graded based on the clearness of the growth inhibition spots, with the clearer spots indicating impressive activity. Impressive growth inhibition against test organisms were seen with the acetone extracts of all medicinal plants under current study, as well as the n-hexane extract of *G. flava*.

In this study, the bioautographic procedure reported before by Begue and Kline [16] and Masoko and Eloff [17] was modified by spraying TLC plates with live bacterial strains without first separating the constituents of the plants extracts with mobile phase solvents. While the non-modified bioautographic procedure affords localization of the polarity of the active compounds [18,19], often solvents used for mobile phases interfere with bacterial growth resulting in poorly developed TLC plates [20]. The interference with bacterial growth in areas where extracts were not loaded was not encountered in this study with the modified procedure. While the interference to bacterial growth by mobile phase solvents was avoided with the modified procedure, the new procedure is limited to only the detection of the presence of bacterial growth inhibition compounds in the plants extracts. Thus, the two methods could be used interchangeably depending on the study objectives with the new method used for activity screening and the previous method used for localization of active compounds on the TLC plate.

CONCLUSION

The leaves of *Barleria dinteri*, *Grewia flava* and *J. lagarinthoides* were found to possess important phytochemicals, antioxidant and antibacterial properties. The findings of the current study regarding the phytochemicals, as well as the antioxidant and antibacterial activities of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* justifies the usage of these plants in African traditional medicine.

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