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Research Article
Effect of Concoction Extraction of the Leaves of *Barleria dinteri* (Oberm), *Grewia flava* (DC) and *Jatropha lagarinthoides* (Sond) on Inherent Antioxidant and Antibacterial Activities

[1]S.S. Gololo, [1]M.A. Mogale, [2]L.J. Shai, [3]N.M. Agyei and [4]P. Masoko

[1]Department of Biochemistry, School of Pathology and Pre-Clinical Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa
[2]Department of Biomedical Sciences, Tshwane University of Technology, Pretoria, South Africa
[3]Department of Chemistry, School of Pathology and Pre-Clinical Sciences, Pretoria, South Africa
[4]Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Polokwane, South Africa

Abstract

Background and Objective: Medicinal plants are often used in mixture (concoctions) for treatment of diseases in traditional medicine. The study aimed at the determination of the effect of concoction extraction of the leaves *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides* on inherent antioxidant and antibacterial activities. **Materials and Methods:** The samples of dried ground leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* were sequentially extracted with n-hexane, dichloromethane, acetone and methanol for both single plant and concoction (mixture of the three plant samples) extractions. The antioxidant and antibacterial activities of the extracts were evaluated through DPPH assay and micro-dilution procedure, respectively with the recording of the EC_{50} (for antioxidant activity) and MIC (for antibacterial activity) values. Obtained values were statistically compared using student t-test (SPSS). **Results:** Extracts obtained with polar solvents, acetone and methanol had higher amounts of total phenolic content. Plants extracts had good antioxidant activity with lower EC_{50} values with the methanol extract of the plant mixture having even much lower EC_{50} value (0.079 mg mL^{-1}) than single plant extracts. The plants extracts also indicated potential antibacterial activity demonstrated by MIC values less than 1 mg mL^{-1}. High potential antibacterial activity was seen in plant mixture extracts with MIC values ranging between 0.023 and 0.188 mg mL^{-1}. **Conclusion:** Generally, the EC_{50} and MIC values of the extracts obtained through concoction extraction were lower than of those obtained by single plant extraction. The results of the current study suggested that concoction extraction of leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* have enhancing effect on inherent antioxidant and antibacterial activities.

Key words: Medicinal plants, antioxidant, antibacterial, concoction extraction, EC_{50}, MIC

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Corresponding Author: S.S. Gololo, Department of Biochemistry, School of Pathology and Pre-Clinical Sciences, Sefako Makgatho Health Sciences University, P.O. Box 235, 0204 Medunsa, Pretoria, South Africa Tel: +27125214372

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Throughout the world, people have been relying on plants for many usages such as shelter, food, clothes and medicine[1]. For medicine, plants extracts had been used as solutions to many problems affecting mankind including treatment of wounds, suspected poisoning and microbial infections. Plants extracts are also used for treatment of complex diseases such as cancer, high blood and diabetes[2]. Often, these medicinal plants are administered as extracts obtained from mixtures of parts from different plants known as concoctions. Herbal mixtures, also known as concoctions have become common features of traditional medicine in South Africa with claims of disease-healing properties attributed to such mixtures[3]. Concoctions are obtained from extracting a mixture of plants, as compared to decoctions (single plant extractions)[4].

Several bioassays have been developed for the determination of the inherent biological properties of the plants extracts. Antioxidant and antimicrobial activities are the most frequently used assays in the screening of medicinal plants for biological properties[5]. Excessive accumulation of oxidative stress and microbial infections have been implicated in a number of disease conditions. Therefore, plants that possess antioxidant and antimicrobial activities are likely to help in alleviation or prevention of many diseases affecting human beings. For the determination of the antioxidant activity of the plants extracts the most used assay is the 2,2-diphenyl-1picrylhydrazil (DPPH) assay[6], while for antibacterial activity the p-iodonitrotetrazolium violet (INT) based micro-dilution method is often used[7]. The strength of antioxidant and antibacterial activities from plants is evaluated through the determination of their effective concentration that gives 50% radical scavenging (EC_{50}) and Minimum Inhibitory Concentration (MICs) against test organism, respectively with lower values indicating potential efficacy[6, 8].

Multi-drug resistance against available treatment agents by microorganisms has necessitated the continuation of the search for antimicrobial products from natural sources, in particular those from plant materials[9]. The three medicinal plants, *B. dinteri*, *G. flava* and *J. lagarinthoides* are amongst those medicinal plants whose mixed leaves are frequently used for traditional medicine in Zebediela, Limpopo province (South Africa) for treatment of diseases associated with bacterial infections. However, the effect that concoction extraction of the leaves of these medicinal plants have on their biological activities in comparison to single plant extraction is not known. Based on the literature, the effect of concoction extraction of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* on their inherent antioxidant and antibacterial activities is reported for the first time.

MATERIALS AND METHODS

Plant material: The leaves of the three medicinal plants were collected from Bolahlakgomo village in Zebediela sub-region of the Limpopo province, South Africa. The identity of the plants was scientifically authenticated by Dr. Egan, a taxonomist and voucher specimen were deposited at the Herbarium of the University of Limpopo (*B. dinteri*-UNIN 11118, *G. flava*-UNIN 11119 and *J. lagarinthoides*-UNIN 11120). After drying at room temperature (25°C) the leaves of the plants were ground to powder.

Extraction: The ground leaves of the three medicinal plants were extracted with hexane, dichloromethane, acetone and methanol in a Serial Exhaustive Extraction (SEE) procedure for both single plant and concoction (mixture of the three plants leaves, designated: BGJ). The extracts were filtered using Whatman filter paper 1, concentrated using a rotavapour and allowed to dry under a stream of air.

Total phenolic content of the extracts: Total phenolic content of the plants' leaf extracts was determined using Folin-Ciocalteu method as described by Abdille *et al*.[9]. Two hundred microlitres of 1:10 diluted sample was added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min of incubation, 800 µL of sodium carbonate (75 g L^{-1}) was added and incubated for 2 h at room temperature. The absorbance was measured at 765 nm using a Multiskan Ascent plate reader (Thermo Labsystems). Tannic acid (0-10 mg mL^{-1}) was used for plotting a standard curve. Results were expressed as percentage of Tannic Acid Equivalents (TAE) per gram dry weight of plant material.

Antioxidant activity

Free radical scavenging activity strength (EC_{50}) of the plant extracts: The strength of the free radical scavenging activity of the extracts was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) assay with vitamin C as standard[9]. Serial dilutions (0-250 µg mL^{-1}) of the plants extracts were prepared to a volume of 150 µL using distilled water. Similar concentrations and volume of ascorbic acid were used as positive control. Then 100 µL 0.1 mM methanolic solution of DPPH was added into the extract solutions and allowed to stand at room temperature for 30 min. The changes in the absorbance of the samples were measured at 550 nm using

Multiskan Ascent plate reader (Thermo Labsystems). The percentage inhibition of the extracts was calculated as follows:

$$\text{DPPH inhibition (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

where, $A_{extract}$ is the absorbance reading of the extracts or the standard sample with DPPH and $A_{control}$ the absorbance of DPPH without extracts or standard. The EC_{50} values of the extracts were obtained from the plots of DPPH inhibition (%) versus extract concentration.

Antibacterial activity

Antibacterial activity strength (MICs) of the plant extracts: To determine Minimum Inhibition Concentration (MIC) of the extracts, 150 µL samples of plants extracts (10 mg mL^{-1}) of the extracts, including the concoction extracts were serially diluted with 150 µL of distilled water in a microplate as described by Eloff[7]. Then 100 µL of test bacterial strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecaelis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were added to the extracts and incubated in a humidified environment. After overnight incubation, 50 µL of 0.2 mg mL^{-1} p-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, USA) was added. The lowest concentration that inhibited the growth of the bacterial strain was taken as the MIC value of the extract with regard to that strain. Gentamycin (0.1%) was used as positive control.

Statistical analysis: The total phenolic content, antioxidant EC_{50} values and MIC values of the extracts were determined. The differences amongst result values in similar extracts of different plants, as well as the plant mixture were assessed for significance using analysis of variance (ANOVA, SPSS). The values were regarded as statistically significant at $p<0.05$.

RESULTS

Total phenolic contents of the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides* as well as the concoction were determined using Folin-Ciocalteu reagent method and the results are shown in Table 1. High amounts of total phenolic compounds were recorded with relatively polar solvents, acetone and methanol in all plants under study. Extracts obtained from the plants mixture (concoction) had higher total phenolic contents than those of single plants. The antioxidant activity of the extracts was evaluated using DPPH assay and the results are shown in Table 2. Lower EC_{50} values against DPPH free radical were obtained with methanol extracts, except in *J. lagarinthoides* were the acetone extract 0.185 mg mL^{-1} had slightly lower value than that of the methanol extract 0.200 mg mL^{-1}. The methanol extract of the plants mixture had even much lower EC_{50} value 0.079 mg mL^{-1} than single plant extracts.

The antibacterial activity of the extracts was evaluated using microplate method and the results are shown in Table 3. In this study, MIC values of less than 1 mg mL^{-1} were considered indicative of potential antibacterial activity. Dichloromethane and acetone extracts of all plants under study showed potential antibacterial activity with MIC values against test organisms ranging between 0.188 and 0.750 mg mL^{-1}. For the n-hexane extracts, potential antibacterial activity was shown only in *B. dinteri*. The dichloromethane and acetone extracts of the plants mixture showed even high potential antibacterial activity against test organisms with much lower MIC values in the range between 0.023 and 0.188 mg mL^{-1}.

Table 1: Total phenolic content of the leaf extracts of *B. dinteri*, *G. flava, J. lagarinthoides* and BGJ (three plants mixture/concoction) expressed as percentage of TAE per gram plant dry weight

Extracts	*B. dinteri*	*G. flava*	*J. lagarinthoides*	BGJ (concoction)
Hexane	0.180±0.169[a]	0.130±0.026[b]	0.095±0.045[c]	0.185±0.146[a]
Dichloromethane	0.200±0.045[a]	0.190±0.103[a]	0.130±0.093[b]	0.245±0.105[c]
Acetone	2.800±0.112[a]	3.200±0.038[b]	2.500±0.124[a]	4.200±0.094[c]
Methanol	3.800±0.038[a]	4.800±0.118[b]	3.600±0.079[c]	5.300±0.188[d]

[a-d]Values with the same letters for the similar extract are not significantly different at $p>0.05$, while those with different letters are significantly different at $p<0.05$

Table 2: EC_{50} (mg mL^{-1}) values of the acetone and methanol extracts of the leaves of *B. dinteri*, *G. flava*, *J. lagarinthoides* and BGJ (three medicinal plants mixture/concoction) against DPPH

	EC_{50} (mg mL^{-1})				
Extracts	*B. dinteri*	*G. flava*	*J. lagarinthoides*	BGJ	Vitamin C
Acetone	0.205±0.108[a]	0.227±0.213[b]	0.185±0.098[c]	0.200±0.201[a]	0.190±0.101
Methanol	0.082±0.065[a]	0.105±0.058[b]	0.200±0.124[c]	0.079±0.028[d]	

[a-d]Values with similar letters for the same extract are not significantly different, while those with different letters are significantly different

Table 3: MIC values (mg mL^{-1}) of the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides*, as well as three plants mixture (BGJ concoction) against test organisms after 24 h incubation

	n-hexane extract (mg mL^{-1})			
Test organism	*B. dinteri*	*G. flava*	*J. lagarinthoides*	BGJ (Concoction)
Escherichia coli	ND	3.00^{a}	6.00^{b}	6.00^{b}
Enterococcus faecalis	ND	0.750^{a}	1.50^{b}	6.00^{c}
Staphylococcus aureus	ND	0.375^{a}	1.50^{b}	6.00^{c}
Pseudomonas aeruginosa	ND	0.375^{a}	1.50^{b}	6.00^{c}
Dichloromethane extract (mg mL^{-1})				
Escherichia coli	0.375^{a}	0.375^{a}	3.00^{b}	0.188^{c}
Enterococcus faecalis	0.750^{a}	0.375^{b}	0.750^{a}	0.188^{c}
Staphylococcus aureus	1.50^{a}	0.750^{b}	1.50^{a}	0.094^{c}
Pseudomonas aeruginosa	0.750^{a}	0.750^{a}	0.750^{a}	0.094^{c}
Acetone extract (mg mL^{-1})				
Escherichia coli	0.188^{a}	3.00^{b}	6.00^{c}	0.094^{d}
Enterococcus faecalis	0.375^{a}	0.375^{a}	0.750^{b}	0.094^{c}
Staphylococcus aureus	0.750^{a}	0.750^{a}	1.50^{b}	0.094^{c}
Pseudomonas aeruginosa	0.375^{a}	0.750^{b}	0.750^{b}	0.023^{c}

$^{a-d}$Values with the same letter for similar extract against the same test organism are significantly not different at $p>0.05$, while those with different letters are significantly different at $p<0.05$ values are averages of triplicates with standard deviation of zero, ND: Not determined, 0.1% of gentamycin was used as positive standard

DISCUSSION

Determination of the phenolic contents of medicinal plants is important as phenolic compounds are known to be a major contributor to the biological activities of medicinal plants[1,6,10]. As expected, higher amounts of total phenolics were found in polar solvents, acetone and methanol. Generally, concoction (BGJ) extracts recorded higher amounts of total phenolics than those of single plant extracts. Determination of the phenolic content of medicinal plants by Folin-Ciocalteu reagent method is based on quantification of the Folin-Ciocalteu reagent reduction activity by the phyto-constituents of the plant extracts and it is therefore not absolute[10]. Polar solvents such as acetone and methanol extract mostly polar compounds from medicinal plants parts, including most phenolic compounds, as such their phyto-constituents are likely to have higher reducing capacity[9]. Phenolic compounds have been reported to contribute to many biological activities found in medicinal plants[10].

Medicinal plants have been shown to possess many biological properties, including antioxidant and antibacterial activities[11]. The antioxidant activity was evaluated only for the acetone and methanol extracts as preliminary investigations showed convincing activity only with the two extracts. The antioxidant strength of the extracts was determined on the basis of their relative EC_{50} values against DPPH[12]. The methanol extract having demonstrated higher total phenolic content had stronger free radical scavenging activity than the acetone extract in *B. dinteri* and *G. flava* with EC_{50} values of 0.082 and 105 mg mL^{-1}, respectively. However, in *J. lagarinthoides* it was the acetone extract with lower total phenolic content than the methanol extract that had stronger free radical scavenging activity than the methanol extract with EC_{50} value of 0.185 mg mL^{-1}. A much lower EC_{50} was recorded for the concoction methanol extract of the three medicinal plants with the value of 0.079 mg mL^{-1}, which indicates even higher antioxidant activity[6,13,14].

In doing antibacterial activity tests, all extracts except the methanol extracts which was not tested were re-dissolved in acetone as it was reported to be non-toxic towards test organisms[15]. The antibacterial strength of the extracts was determined on the basis of their MIC values against test organisms with lower values indicative of potential antibacterial activity[7,8,16]. The MICs were not determined for the n-hexane extract of *B. dinteri* as it did not show any antibacterial activity during preliminary investigations. In this study, MIC values of less than 1 mg mL^{-1} were considered to be indicative of good antibacterial strength. With regard to single plant extracts obtained using dichloromethane, MIC value of 0.375 mg mL^{-1} were recorded for *B. dinteri* against *E. coli*, as well as for *G. flava* against *E. coli* and *E. faecalis*. Dichloromethane extracts of the concoction gave even lower MIC values ranging between 0.188 and 0.094 mg mL^{-1} amongst test organisms. Regarding the acetone extracts, the MIC value of 0.188 mg mL^{-1} was recorded for *B. dinteri* against *E. coli*, whereas MIC values of concoction extracts were much lower ranging between 0.094 and 0.023 mg mL^{-1} amongst test organisms. The recorded lower MIC values for concoction extracts compared to single plant extracts suggest higher antibacterial strength by concoction extracts. However, an exception in the trend of the results was observed in the n-hexane extracts whereby lower MIC values

(0.750 mg mL^{-1} against *E. faecalis*, 0.375 mg mL^{-1} against *S. aureus* and *P. aeruginosa*) were recorded for *B. dinteri* compared to higher MIC values (6.00 mg mL^{-1}) by concoction extracts.

The results of the current study are in agreement with findings of similar previous study involving different plants. Iwalokun *et al.*[17] in their study showed that the concoction extract of *Ocimum gratissimum* and *Terminalia avicennoides* have shown greater shegellocidal activity against *Shigella dysenteriae*, *Shigella sonnei*, *Shigella boydii* and *Escherichia coli*. The concoction showed higher effectiveness as compared to single plant extracts of either *O. gratissimum* or *T. avicennoides*. However, in the same study, the concoction of the same two plants had less activity than decoction extract of *T. avicennoides* against *Shigella flexneri*. This selective enhancement of on biological activity through concoction extraction as seen in the current study and previous study[17], necessitate more studies on medicinal plants used as concoctions in traditional medicine.

CONCLUSION

The findings of the current study indicate enhancement of the antioxidant and antibacterial activities of the leaves of *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides* through concoction extraction with the exception of the n-hexane extract. This enhancement effect could be due to higher amounts of phenolic compounds found in the extracts obtained by concoction extraction procedure.

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