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Full Length Research Paper

Effect of seasonal changes on the quantity of phytochemicals in the leaves of three medicinal plants from Limpopo province, South Africa

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The study was carried out to investigate the effect of seasonal changes on the quantity of phytochemicals in leaves of three medicinal plants: *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinhoidea*, collected from Limpopo province in South Africa. Three medicinal plants were collected during different seasons of the year and phytochemicals were quantified on plant samples collected in each season. Alkaloids and tannins were in high amounts during colder seasons (autumn and winter) in all plants, whereas flavonoids were in high amounts during warmer seasons (spring and summer). In addition, saponins were in high amounts during warmer seasons in *G. flava* and *J. lagarinhoidea*, while no significant seasonal difference was recorded in *B. dinteri*. Furthermore, simple phenols were in high amounts during autumn in *G. flava*, while in high amounts during summer in *J. lagarinhoidea* with no significant difference amongst seasons in *B. dinteri*. The findings of the study indicate that seasonal changes affected the quantity of inherent phytochemicals in the leaves of three medicinal plants under current study in a phytochemical specific manner.

Key words: *Barleria dinteri*, *Grewia flava*, *Jatropha lagarinhoidea*, phytochemicals, seasonal changes, quantitative

INTRODUCTION

World-wide, medicinal plants continue to be relied upon as solutions to health problems (Verma and Singh, 2008). 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 (Van Wyk et al., 1997).

This rural dependence 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 (Motamedi et al., 2010).

Medicinal plants synthesize a number of biological

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constituents that are needed for the plant's survival and its protection against predators and insects. Such biological constituents needed for the survival of plants are referred to as secondary metabolites or phytochemicals. The secondary metabolites are an extremely diverse group of compounds not only chemically but also in their functions where they are commonly used in agriculture and drug discovery (Yadav and Agarwala, 2011). Phytochemicals such as alkaloids, flavonoids, saponins and tannins are known to have a definite physiological effect on the human body that include antioxidant, antibacterial, anti-cancer and anti-inflammatory activities (Craig, 1999).

Phytochemical composition of medicinal plants is known to be affected by a number of environmental factors including altitude, soil type and change of season (Nchabeleng et al., 2012; Jayanthi et al., 2013; Odjegba and Alokolaro, 2013). Effect of seasonal changes on the formation of plant secondary metabolites is influenced by responses to a variety of season specific pathogens (Figueiredo et al., 2008). This effect affords plants, unlike other organisms, to survive different seasonal conditions without hibernation. Seasonal changes expose plants to different temperature levels (including extreme levels) that have an effect in their phytochemical compositions, with volatile compounds being the most affected (Usano-Alemany et al., 2014). Determination of the seasonal effect on plant phytochemical compositions provides knowledge on the time/season of harvest of individual plant species that afford optimum concentration of active ingredients (Kale, 2010).

The leaves of the three medicinal plants; *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides*, are widely used in the Limpopo province of South Africa in traditional medicine for treatment of microbial infections, intestinal tumors and stomach cramps (Gololo, personal communication). Preliminary analyses in our laboratory have revealed that the leaves of the three medicinal plants possess important phytochemicals such as alkaloids, flavonoids, saponins and tannins. The leaves of these plants are collected throughout the year for medicinal usage. In South Africa, four seasons with different rainfall patterns and average temperatures are found within a year, namely: autumn, winter, spring and summer. However, there is lack of information on the effect that changes of seasons have on the quantity of the inherent phytochemicals of the three medicinal plants under current study. This information will be important in the determination of the suitable harvesting season of the three medicinal plants for usage in traditional medicine.

MATERIALS AND METHODS

Plant

The leaves of *B. dinteri* (voucher specimen: UNIN 11118), *G. flava* (voucher specimen: UNIN11119) and *J. lagarinthoides* (voucher specimen: UNIN 11120) were collected from Bolahlakgomo village

in Zebediela sub-region, Limpopo province (South Africa) during the four annual seasons [autumn (March 2012, average temperature 13; 24°C, average rainfall \pm 50 mm), winter (June 2012, average temperature 0; 19°C, average rainfall \pm 5 mm), spring (September 2012, average temperature 8; 26°C, average rainfall \pm 15 mm) and summer (December 2012, average temperature 15; 28°C, average rainfall \pm 75 mm)], temperatures and rainfall levels data were obtained from Worldweather online (worldweatheronline.com) for the period 2000 to 2012. The leaf samples were dried at room temperature, ground to powder and stored in the dark until further usage.

Phytochemical analyses

The three medicinal plants were analysed for the amounts of simple phenols, flavonoids, tannins, alkaloids, and saponins, using standard procedures for the quantitative analysis of these phytochemicals already reported in the literature (Obadoni and Ochuko, 2001; Hussain et al., 2011; Krishnaiah et al., 2009).

Quantitative determination of alkaloids

Five grams of the ground leaves were weighed into separate 250 ml beaker, followed by addition of 200 ml of 20% acetic acid in ethanol. The mixtures were covered and allowed to stand at room temperature in the dark for 4 h. The mixtures were then filtered and the extracts concentrated by evaporating one quarter of the volume over a boiling water bath. Concentrated ammonium solution was then added drop wise to the extract until precipitation was completed. The solution was allowed to settle and the precipitates collected by filtration, dried and then weighed. The resultant dry mass was expressed as a percentage of the mass of the starting plant material (Obadoni and Ochuko, 2001).

Quantitative determination of flavonoids

Five grams of the ground leaves were weighed into a 250 ml Erlenmeyer flask and 100 ml of 80% aqueous methanol added and shaken for 4 h at room temperature using an electric shaker (Labotec, SA). The mixtures were then filtered and the filtrates evaporated to dryness over a boiling water bath before weighing. The resultant dry mass was expressed as a percentage of the mass of the starting plant material (Hussain et al., 2011).

Quantitative determination of (simple) phenols

Five grams of the ground leaves of each medicinal plant were weighed into 250 Erlenmeyer flask and extracted twice for 4 h with 100 ml *n*-hexane. The resultant *n*-hexane extracts were filtered and 50 ml diethyl ether was added to the fat-free residues. The mixtures were then heated for 15 min, cooled to room temperature and filtered twice into a separating funnel. Then, 50 ml of 10 % sodium hydroxide solution was added to the mixtures in separating funnels and shaken to separate the aqueous layer from the organic layer. The organic layers were then washed three times with 25 ml of distilled water and the pooled aqueous layers were titrated to pH 4.0 with 10% hydrochloric acid solution. Then, 50 ml of dichloromethane was added and mixtures transferred to new separating funnel. Finally, the organic layer was collected, dried and weighed. The resultant dry mass was expressed as a percentage of the mass of the starting plant material (Hussain et al., 2011).

Table 1. Seasonal quantities (% w/w-dry plant material) of phytochemicals from the leaves of *B. dinteri*.

Phytochemical group	Autumn	Winter	Spring	Summer
Alkaloids	5.20±0.310 ^a	6.73±0.351 ^b	1.71±0.169 ^c	1.53±0.045 ^c
Flavonoids	17.9±0.451 ^a	21.4±0.153 ^b	23.3±1.01 ^c	25.1±0.404 ^d
Phenols (simple)	1.17±0.252 ^a	1.29±0.121 ^a	1.32±0.537 ^a	1.46±0.097 ^a
Saponins	10.5±0.475 ^a	11.1±1.39 ^a	10.8±0.370 ^a	11.4±0.361 ^a
Tannins	28.6±0.666 ^a	24.5±2.86 ^b	12.4±0.529 ^c	12.2±0.200 ^c

^{a,b,c,d} Mean values with the same letter per phytochemical group (in rows) are not significantly different ($p > 0.05$), while those with different letters per phytochemical group are significantly different ($p < 0.05$)

Table 2. Seasonal quantities (% w/w-dry plant material) of phytochemicals from the leaves of *G. flava*.

Phytochemical group	Autumn	Winter	Spring	Summer
Alkaloids	10.4±0.208 ^a	6.66±0.401 ^b	1.61±0.100 ^c	1.75±0.230 ^c
Flavonoids	10.8±0.265 ^a	15.3±0.721 ^b	21.3±1.12 ^c	24.5±0.656 ^d
Phenols (simple)	1.28±0.146 ^a	1.04±0.229 ^a	0.808±0.095 ^b	0.695±0.038 ^b
Saponins	5.04±0.584 ^a	6.33±0.804 ^a	12.2±0.404 ^b	14.8±0.473 ^c
Tannins	23.7±0.702 ^a	23.1 ±0.306 ^a	13.9±1.86 ^b	12.9±1.70 ^b

^{a,b,c,d} Mean values with the same letter per phytochemical group (in rows) are not significantly different ($p > 0.05$), while those with different letters per phytochemical group are significantly different ($p < 0.05$).

Quantitative determination of saponins

Five grams of the powdered leaves were weighed and dissolved in 100 ml of 20% ethanol. The suspensions were then heated with continuous stirring over a water bath (55°C) for 4 h. The mixtures were then filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrated extract was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether added, with the mixture shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. Then, 30 ml of *n*-butanol was repeatedly added to the recovered aqueous layer and washed twice with 5% aqueous sodium chloride. The resultant solution was then evaporated over a boiling water bath, dried in an oven and then weighed. The resultant dry mass was expressed as a percentage of the mass of the starting plant material (Obadoni and Ochuko, 2001).

Quantitative determination of tannins

Five grams of grounded leaves were weighed and added into 50 ml reagent bottles followed by addition of 50 ml distilled water. The resultant mixtures were then stirred and filtered into 50 ml volumetric flasks. The resultant filtrates were then made to 50 ml with distilled water, following which 5.0 ml aliquots of the filtrates were pipetted into different test tubes and mixed with 2 ml of 0.1 M hydrochloric acid in 0.008 M potassium ferric cyanide. The absorbance of the mixtures were measured at 395 nm on a Carry 50 spectrophotometer (Varian Technologies, USA) and converted into concentrations (mg/ml) using a tannic acid standard curve. The yield was then expressed as a percentage of the mass of the starting material (Krishnaiah et al., 2009).

Statistical analysis

Experimental results were expressed as means ± standard

deviations ($n = 3$, from 3 independent experiments). Differences between phytochemical quantities of the plant leaf samples were determined using analysis of variance (ANOVA, SPSS). The values were regarded as statistically significant at $p < 0.05$.

RESULTS

Seasonal quantitative phytochemical analysis of the three medicinal plants showed that the leaves of the three medicinal plants had different amounts of alkaloids, flavonoid, phenols, saponins and tannins during various seasons of the year. The results are shown in Tables 1, 2 and 3 for *B. dinteri*, *G. flava* and *J. lagarinhoides*, respectively.

Alkaloids and tannins were recorded in high amounts during colder seasons [autumn (March) and winter (June)] for all plants, whereas flavonoids were in high amounts during warmer seasons [spring (September) and summer (December)]. Saponins were in high amounts during warmer seasons for *G. flava* and *J. lagarinhoides*, while no significant seasonal difference was recorded for *B. dinteri*. Simple phenols were in high amounts during colder seasons for *G. flava*, while in high amounts during summer for *J. lagarinhoides*. There was no significant seasonal difference in the amounts of simple phenols for *B. dinteri*.

DISCUSSION

Determination of the effect of seasonal changes on the quantity of plants phytochemicals is an important step to

Table 3. Seasonal quantities (% w/w-dry plant material) of phytochemicals from the leaves of *J. lagarinhoides*.

Phytochemical group	Autumn	Winter	Spring	Summer
Alkaloids	2.73±0.045 ^a	5.88±0.658 ^b	1.45±0.026 ^c	1.88±0.093 ^c
Flavonoids	18.7±1.25 ^a	14.4±1.33 ^b	30.2±2.80 ^c	23.9±0.643 ^d
Phenols (simple)	1.15±0.130 ^a	1.04±0.206 ^a	1.32±0.537 ^a	1.67±0.120 ^b
Saponins	7.41±1.39 ^a	6.95±1.16 ^a	11.8±1.00 ^b	13.9±1.49 ^c
Tannins	23.8±0.306 ^a	24.3±2.88 ^a	14.3±2.66 ^b	13.6±0.987 ^b

^{a,b,c,d} Mean values with the same letter per phytochemical group (in rows) are not significantly different ($p > 0.05$), while those with different letters per phytochemical group are significantly different ($p < 0.05$).

establish the suitable harvesting season with high amounts of phytochemicals. The dose dependent biological activities of medicinal plants have been reported before (Dilipkumar et al., 2011; Moyo and Mukanganyama, 2015). The results of the current study showed a variation in the seasonal quantities of phytochemicals of the three medicinal plants investigated. The results also indicate that medicinal plants may have high accumulation of different phytochemicals during different seasons, as seen in the case of alkaloids and tannins been accumulated in high amounts during colder seasons while flavonoids were accumulated in high amounts during warmer seasons for all medicinal plants under current study.

The results of the current study are in agreement with the findings of the previous studies, although with different plant species. In this regard, the leaves of *Barleria prionitis*, *Boerhavia diffusa*, *Citrillus colocynthis* and *Grewia tenax* were reported to have different yields of total alkaloids and polyphenols in different seasons, with the Indian summer (April-June) showing the highest yield compared to winter (December-February) and rainy season (July-September) (Sahoo et al., 2012). In a separate study, total alkaloids and polyphenols were high during summer season in *Convoliulus microphyllus*, whereas the same phytochemical groups were high during winter in *Datura metel* (Kale, 2010). The differences in the seasonal phytochemical quantities of the leaves of medicinal plants were earlier reported to cause seasonal variation in their biological activities (Ncube et al., 2011). The four seasons during which plant samples were collected are characterised by different average temperatures and rainfall patterns. These environmental factors are already reported to have an effect on the quantities of phytochemicals in certain plants (Kale, 2010; Usano-Aleman et al., 2014).

Conclusion

With the findings of the current study, it could be concluded that the leaves of the three medicinal plants: *B. dinteri*, *G. flava* and *J. lagarinhoides* possess important phytochemicals whose quantity differs with

seasonal changes. Since different phytochemicals possess different biological activities and they are accumulated in high amounts during different seasons, therefore one plant species may be harvested during different seasons (depending on availability) for treatment of different diseases. There is however, a need to use analytical techniques such as high performance liquid chromatography to supplement the data obtained in this study using standard methods.

Conflict of interests

The authors have not declared any conflict of interests.

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