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Phytopharmacological Assessment of Some Medicinal Plants of Thal Desert of Pakistan

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GY, ZK conducted experiment. Author AA worked for writing and proof reading. All authors read and approved the final manuscript.

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ABSTRACT

The presence of secondary metabolites and various ions in a plant determines its phytophamacological potential. Desert plants are adapted to stressful environmet by synthesizing secondary metabolites and ions accumulation as osmoticum.The present study was conducted to evaluate the pharmacological potential of Thal desert plants in term of their metabolites and nutrient ions concentrations. Five specimens of seasonally available herbs and three of trees of Thal desert plants were colected. After collection specimens were analysed for alkaloids, terpenoids, tannins, sugar and ion contents. The data were analyzed statistically and means were compared by Duncan's Multiple Range Test. Among the herbs *Panicum antidotale* root showed highest terpenoid, K^+ ion and Ca^+ ion contents. The herb *Aerva javanica* stem showed lowest alkaloid, tannin, soluble sugar, phosphorus, potassium and calcium contents. Among trees specimens, *Tamarix aphylla* leaves showed highest soluble sugar, phosphorous, potassium and calcium contents. Stem of *Acacia modesta* showed the lowest alkaloid, terpenoid, phosphorous and potassium contents.

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1. INTRODUCTION

The urge to evaluate the medicinal potential of plants has increased due to finding the sources of alternative medicines in recent era. Majority of the plants contain natural antioxidants and phytochemicals being utilized in pharmaceutical industry [1]. Globally, the demand of medicinal plants is increasing due to their being easy accessible, few side-effects and sometimes their being the sole source of medicine [2]. Thus, the research on phytochemicals is valuable in the field of medicine and nutrition.

Desert plants produce many such phytochemicals by secondary metabolism which help plants for survival under drought conditions of desert. The phytochemicals obtained from these desert plants are used in drugs [3]; nutrition [4] and in defense from predation [5]. The most common among these phytochemicals are saponin, alkaloids, flavonoids, tannins and phenolics. Antioxidant activity of plants is mainly due to phenolic compounds [6]. Phenolics have many biological activities including anti-microbial,I mmune-stimulating agents, anti-carcinogenic, antiallergenic, cardio-protective [7]. Alkaloids belong to group of chemical compounds having nitrogen as basic atom. This group also includes compounds having neutral [8] and weak acidic nature [9]. They play role in plants defense against other plants, micro-organisms, insects and herbivores due to allopathic characteristics [10]. Therefore, are used for medicinal purposes. In humans alkaloids affect nervous system .Basically these work as chemicals transmitters and have also antibiotic activity [11]. Flavonoids are considered as pigments that give colors [12,13]. They are used in foods [14].

There are four types of deserts present in Pakistan these are Cholistan, Thar, Nara and Thal desert. The Thal desert is present in province Punjab of Pakistan between 31° 10' north and 71° 30' east. This subtropical region is sandy and spread over 190 miles and with its coverage of 70 miles . During summer temperature of the desert Cholistan reaches up to 50 ºC due to which environment becomes arid. The humidity level at Cholistan desert is 35 to 65% [15]. The annual rain fall is about 150 to 350 millimeter [16]. The vegetation of the Thal desert consists of mainly shrubs, trees and grasses. The main vegetation present at Thal desert include *Capparis decidua* (Karir), *Tamarix aphylla* (Farash), *Capparis aphylla* (Karir), *Prosopis cineraria* (Jand), *Calligonum polygonoides* (Phog), *Solvadora oleoides* (Van), *Acacia jacquemontii* (Babble acacia), *Haloxylon recurvum* (Lana), [17], *Prosopis spicigera* (Jand) and *Zizyphus nummularia* (Malla).

2. MATERIALS AND METHODS

2.1 Specimens collection

After an initial survey of Thal desert, plants were collected , labelled and brought to lab. After washing,drying and grinding the samples were analysed for various biochemicals.

2.2 Alkaloid Analysis

For the alkaloid contents analysis, Harborne [18] method was used. The method is named as gravimetric method. 0.50 gram of finely ground plant material was put in test tubes. The plant material was soaked in 10% acetic acid solution in ethanol in the ratio of 1:10 (10%). Then the mixture was placed at orbital shaker overnight at 150rpm. After it the mixture was filtered by using Whatman No.42 filter paper. Then the precipitation was done by drop by drop addition of concentrated aqueous NH_4OH. The precipitate was obtained by using weighed filter paper. The filter paper washed with 1% Ammonia solution. And oven dried at 80ºC for half hour. Then the Alkaloid contents was calculated as percentage of weighed sample.

2.3 Terpenoid Analysis

Finely ground plant material (2g) was taken in test tubes. Then 50 ml of 95% ethanol was added and placed at orbital shaker overnight at 50 rpm. Then filtration was done by using filter paper. After it the terpenoids were precipitated by adding drop by drop petroleum ether. After it filtered by using weighed paper and placed in oven for half hour at 80ºC and total terpenoid contents were determined [19].

2.4 Tannin Analysis

For the analysis of Tannin Swain's method [20] was used. 0.2 gram of powdered plant material (Leafs, stem and root) was weighed at electric balance. And put in 50 ml beaker. The plant material was soaked in 20 ml of 50% methanol and covered with paraffin layer to prevent

evaporation. Then placed in water bath for 1 hour at 77-80ºC and stirred with glass rod to prevent lumping. In a 100 ml volumetric flask extract was filtered by using double layered Whatman No.1 filter paper and rinsed with 50% methanol solution. And level was made upto 100ml by adding distilled water and mixed well. Then from it 1ml sample was taken in 50 ml volumetric flask, and then 2.5 ml Folin-Denis reagent was added. After it 17% 10 ml of Na_2CO_3 was added and made upto mark by adding distilled water and placed this for 20 mints for the development of bluish-green color. Then the standards from tannic acid solution were prepared in range of 0-10 ppm and treated as 1 ml sample above. The absorbance was taken by using 21D spectrophotometer at 760 wavelengths and percentage was calculated.

2.5 Sugar Contents Analysis

For the purpose of soluble sugars contents determination a method described by [21] was used. 0.1 g of powdered plant sample was weighed at electric balance. Plant material was soaked in 10 ml of ethanol and placed at orbital shaker for overnight extraction. Then the extract was filtered by using filter paper. 2 ml of 50% phenol solution was added in extract. Then 2 ml concentrated H_2SO_4 added in this mixture. The sample was put in plastic bottles. The mixture was placed in fridge for 40 mints at 4ºC. After it the mixture was placed at any dry place. And absorbance was taken by using spectrophotometer.

2.6 Digestion of Plant Material

0.10 g of finely ground plant material (leaf, stem and root) was weighed at electronic balance. Put in the digestion flask and 3 ml of concentrated $H_2SO_4$was added in plant material then placed in the digestion block for 30 mints at 100ºC. Then temperature increased up to 350ºC and allowed sample to digest. After 30 minutes digestion flasks were taken out from digestion block and allowed to cool. When mixture becomes cold then 4 ml of H_2O_2 was added in each flask the mixture turned yellow. These flasks were again placed in digestion block and again treated with same temperature. After 1 hour some samples become colorless while some samples were retaining light yellow color. The flask was again removed from digestion block and allowed to cool down. When mixture becomes cold then 4 ml of H_2O_2 was added in flasks retaining light yellow color. By the addition of H_2O_2 all solution became colorless. After it the colorless extract was filtered by using filter paper and volume of the extract was made up to 50 ml by adding distilled water. The extract was stored to analyze calcium, potassium and phosphorus [22].

2.7 Calcium Determination

For the calcium determination flame photometer was used. Standards were prepared in range of 0-10ppm. The knob of Flame photometer was fixed at Ca^+ ion. The values of standards were calculated at flame photometer and standard curve was drawn. Then one by one values of digestion mixture for each plant sample was calculated. These values were compared with standard curve separately and calcium contents were calculated.

2.8 Potassium Determination

For the purpose of potassium determination flame photometer was used. First of all standards were prepared in range of 0-10ppm. The flame photometer was set down and knob was fixed at potassium. The values of standard were taken at flame photometer and a standard curve was prepared by using these values. Then one by one values of digestion mixture was taken. Then the value of each sample, potassium contents was compared with standard curve and total amount of potassium was calculated for each sample mixture.

2.9 Phosphorous Determination

Phosphorous contents weres calculated by using spectrophotometer. 2ml from digestion mixture was taken from each plant sample separately. Then they were treated by the addition of 2 ml Barton reagent. The volume of the sample was made up to 50 ml by adding distilled water. The sample was allowed to stand for 1 hour at room temperature. Standards for Phosphorous were prepared in rang of 0-10ppm. The absorbance of standards was calculated by using spectrophotometer. The standard curve was drawn by using these values. The absorbance of each plant sample was calculated one by one. This absorbance was compared with standard curve and total Phosphorouscontents was determined.

2.10 Barton Reagent

For the preparation of Barton reagent two solutions was prepared. Solutions are:

1. Solution 1: A solution of Ammonium molybdate was prepared by mixing 25 gm of Ammonium molybdates in 400 ml of distilled water.
2. Solution 2: A second solution of Ammonium metavanadate was prepared in which 125 gm of Ammonium metavanadate was mixed in 300 ml boiling water. The solution was allowed to cool down. Then 250 ml concentrated HNO_3 was added. Then the solution was again allowed to cool at room temperature.At the end solution 1 and 2 were mixed down. And the level of solution was made up to 1000 ml by adding distilled water. The reagent was stored at room temperature.).

2.11 Statistical Analysis

Statistical analysis was done by using one way ANOVA. Degree of freedom, MS value, significant and non-significant differences were found. Duncan's multiple range test (p=0.05) was applied to find out the standard deviation, Least significant difference and mean values. [23].

3. RESULTS

3.1 Alkaloid Contents of Herbs

The resulted data of The percentage differences from standard difference from standard value were as for alkaloid contents given in (Table 1). The leaves pf *Aerva javanica* showed maximum Alkaloid contents (0.035 mg/g) and minimum (0.004 mg/g) was found in *Aerva javanica* stem. The comparison of mean values showed non-significant differences among *Aerva javanica* stem (0.004 mg/g), *Aerva javanica* leaves (0.035 mg/g), , *Panicum antidotale* stem (0.032 mg/g) and *Panicum antidotale* root (0.008 mg/g) except *Panicum antidotale* leaves (0.023 mg/g) which showed significant differences. *Aerva javanica* stem was considered as standard. The percentage differences from standard were as *Aerva javanica* leaves (775.00%), *Panicum antidotale* stem (700.00%), *Panicum antidotale* leaves (475.00%) and *Panicum antidotale* root (100.00%) for Alkaloid contents is more than standard.

3.2 Terpenoid Contents of Herbs

The resulted data of percentage differences from standard difference from standard value were as for terpenoid contents of herbs and their parts is given in Table 1. *Panicum antidotale* root showed maximum terpenoid contents (0.032 mg/g) and *Aerva javanica* leaves showed minimum (0.002 mg/g) terpenoid contents. The mean comparison showed non-significant differences among *Aerva javanica* stem (0.005 mg/g), *Aerva javanica* leaves (0.003mg/g), *Panicum antidotale* stem (0.027 mg/g) and *Panicum antidotale* root (0.032 mg/g) while *Panicum antidotale* leaves (0.023 mg/g) showed significant differences. *Aerva javanica* stem was considered as standard. The concentration in *Aerva javanica* leaves was 40.00% less than that of standard while more values were recorded in *Aerva javanica* root (100.00%), *Panicum antidotale* stem (360.00%), *Panicum antidotale* leaves (360.00%) and *Panicum antidotale* root (540.00%).

3.3 Tannin Contents of Herbs

The resulted data of the percentage differences from standard difference from standard value were as for tannin contents in herbs are given in Table 1.The maximum concentration of tannin contents (0.237 mg/g) was recorded in stem of *Panicum antidotale* and minimum (0.082 mg/g) was noted in *Aerva javanica* stem. The mean comparison showed non-significant differences among *Aerva javanica* stem (0.082 mg/g), *Aerva javanica* leaves (0.213 mg/g), *Panicum antidotale* stem (0.237 mg/g), *Panicum antidotale* leaves (0.193 mg/g) and *Panicum antidotale* root (0.080 mg/g). *Aerva javanica* stem was considered as standard. The percentage differences from standard were as *Aerva javanica* leaves (163.75%), *Panicum antidotale* stem (189.024%) and *Panicum antidotale* leaves (135.366%) while percentage of *Panicum antidotale* root (2.439%) is less than standard.

3.4 Soluble Sugar Contents of Herbs

The data of the soluble sugar contents of herbs are given in Table 1.The maximum soluble sugar contents (20.453 mg/g) were found in *Aerva javanica* leaves and *Aerva javanica* stem (10.246 mg/g) showed minimum soluble sugar contents. The mean comparison showed significant differences among *Aerva javanica* stem (10.264 mg/g), *Aerva javanica* leaves (20.453 mg/g), *Panicum antidotale* stem (15.241 mg/g), *Panicum antidotale* leaves (12.946 mg/g) and *Panicum antidotale* root (14.047 mg/g). *Aerva javanica* stem was considered as standard. The percentage differences from standard were as *Aerva javanica* leaves (99.269%), *Panicum antidotale* stem (48.489%), *Panicum antidotale* leaves (26.130%) and *Panicum antidotale* root (36.857%).

3.5 Alkaloid Contents of Trees

The resulted data of the alkaloid contents are given in Table 2. The stem of *Tamarix aphylla* showed maximum alkaloid contents (0.064 mg/g) and *Acacia modesta* stem showed minimum (0.014 mg/g) alkaloid contents. The mean comparison showed significant differences of *Tamarix aphylla* stem (0.064 mg/g) while *Acacia modesta* stem (0.014 mg/g) and *Acacia modesta* leaves (0.019 mg/g) showed non-significant differences.*Tamarix aphylla* stem was considered as standard. The percentage differences from standard were as *Acacia modesta* stem (78.125%) and *Acacia modesta* leaves (70.312%) for alkaloid contents.

3.6 Terpenoid Contents of Trees

The data of the terpenoid contents of trees are shown in Table 2. The stem of *Tamarix aphylla* revealed maximum terpenoid contents (0.024 mg/g) and *Acacia modesta* stem showed minimum (0.008 mg/g) terpenoid contents. The differences were non-significant among *Tamarix aphylla* stem (0.024 mg/g) and *Acacia modesta* leaves (0.016 mg/g)except *Acacia modesta* stem (0.008 mg/g).*Tamarix aphylla* stem was considered as standard. The percentage differences from standard were as *Acacia modesta* stem (66.667%) and *Acacia modesta* leaves (33.333%) for terpenoid contents were less than standard.

3.7 Tannin Contents of Trees

The data regarding the tannin contents in trees are depicted in Table 2. The maximum Tannin contents (0.295 mg/g) were found in stem of *Acacia modesta* while *Tamarix aphylla* leaves showed minimum (0.226 mg/g) tannin contents. The comparison of mean values showed non-significant differences among *Acacia modesta* stem(0.295 mg/g), *Tamarix aphylla* leaves (0.226 mg/g) and *Tamarix aphylla* stem (0.229 mg/g). *Tamarix aphylla* stem was considered as standard. The percentage differences from standard were as *Tamarix aphylla* leaves (1.310%) for Tannin contents is less than standard while *Acacia modesta* stem had 28.820% more value than standard.

3.8 Soluble Sugar Contents of Trees

The data pertaining to soluble sugar contents in trees are given in Table 2. In leaves of *Tamarix aphylla* contents of soluble sugars were maximum (20.085 mg/g) and *Tamarix aphylla* stem revealed minimum (16.35mg/g) soluble sugar contents.The mean comparison showed significant differences among *Tamarix aphylla* stem (16.35 mg/g), *Tamarix aphylla* leaves (20.085 mg/g) was was taken as standard. The more percentage values from standard were of *Tamarix aphylla* leaves (22.844%) and *Acacia modesta* stem (11.174%).

3.9 Nutrients of Herbs

The data for phosphorus,potassium and calcium contents of herbs are given in Table. 3. The maximum concentration of phosphorus (2.234 mg/g) was in Leaves of *Panicum antidotale* while minimum (1.532 mg/g) phosphorous contents was found in stem of *Aerva javanica*. The mean comparison showed non-significant differences among *Aerva javanica* stem (1.532 mg/g), *Aerva javanica* leaves (1.748 mg/g), *Panicum antidotale* stem (1.907 mg/g), *Panicum antidotale* leaves (2.234 mg/g) and *Panicum antidotale* root (1.728 mg/g). Maximum Potassium ion contents (5.013 mg/g) was in *Panicum antidotale* root and minimum (1.098 mg/g) in stem of *Aerva javanica*. The comparison of means showed on-significant differences among *Aerva javanica* stem (1.098 mg/g), *Aerva javanica*leaves (1.120 mg/g) and *Panicum antidotale* stem (1.127 mg/g) while *Panicum antidotale* leaves (2.820 mg/g) and *Panicum antidotale* root (5.013 mg/g).. Maximum Calcium contents (6.159 mg/g) was in *Panicum antidotale* root and minimum (4.403 mg/g) in *Aerva javanica* stem. Significant differences were among means of *Aerva javanica* stem (4.403 mg/g), *Aerva javanica* leaves (4.934 mg/g), *Panicum antidotale* stem (5.707 mg/g), *Panicum antidotale* leaves (5.318 mg/g) and *Panicum antidotale* root (6.159 mg/g).

3.10 Nutrients of Trees

The data for phosphorus,potassium and calcium contents of trees are given in Table 4. In *Tamarix aphylla* leaves the concentration of phosphorus was maximum (0.929 mg/g) while minimum (0.415 mg/g) was found in *Acacia modesta* stem. The mean comparison showed significant differences among *Tamarix aphylla* stem (0.846 mg/g), *Tamarix aphylla* leaves (0.929 mg/g) and *Acacia modesta* stem (0.415 mg/g). Maximum Potassium ion contents (4.396 mg/g) was in *Tamarix aphylla* leaves while minimum (1.117 mg/g) was in *Acacia modesta* stem. The mean

Table 1. Mean comparison of alkaloid, terpenoids, tannins and total soluble sugar contents (mg/g) in herbs of Thal desert

Plant	Alkaloid (mg/g) LSD=0.008	Terpenoid (mg/g) LSD=0.004	Tannin (mg/g) LSD=0.103	SolubleSugar (mg/g) LSD=0.832
Aerva javanica(S)	0.004±0.002 (d)	0.005±0.002 (d)	0.082±0.092 (b)	10.264±0.351 (e)
Aerva javanica(L)	0.035±0.007 (a) (-775.000)	0.003±0.002 (d) (40.000)	0.213±0.029(a) (-163.75)	20.453±0.446(a) (-99.269)
Panicum antidotale(S)	0.032±0.007 (a) (-700.000)	0.027±0.002 (a) (-360.000)	0.237±0.046(a) (-189.024)	15.241±0.324(b) (-48.489)
Panicum antidotale(L)	0.023±0.004 (b) (-475.000)	0.023±0.002 (b) (-360.000)	0.193±0.020(a) (-135.366)	12.946±0.440(d) (-26.130)
Panicum antidotale(R)	0.008±0.003 (cd) (-100.000)	0.032±0.003 (a) (-540.000)	0.080±0.090(b) (2.439)	14.047±0.652(c) (-36.857)

Means showing different letters differed significantly p ≤ 0.05, LSD= Least significant difference, S= Stem, L= Leaf, R= Root

Table 2. Mean comparison of alkaloid, terpenoids, tannins and total soluble sugar contents (mg/g) in trees of Thal desert

Plant	Alkaloid (mg/g) LSD=0.013	Terpenoid (mg/g) LSD=0.007	Tannin (mg/g) LSD=0.069	SolubleSugar (mg/g) LSD=1.231
Tamarix aphylla(S)	0.064±0.012 (a)	0.024±0.003 (a)	0.229±0.049 (a)	16.35±0.792 (c)
Tamarix aphylla(L)	0.037±0.005 (b) (42.187)	0.018±0.003 (ab) (25.000)	0.226±0.029 (a) (1.310)	20.085±0.259 (a) (-22.844)
Acacia modesta(S)	0.014±0.004 (c) (78.125)	0.008±0.004 (c) (66.667)	0.295±0.019 (a) (-28.820)	18.177±0.667 (b) (-11.174)

Means showing different letters differed significantly p ≤ 0.05, LSD= Least significant difference, S= Stem, L= Leaf, R= Root

Table 3. Mean comparison of Phosphorus, Potassium and and Calcium contents (mg/g) in herbs of Thal desert

Plants	Phosphorous (mg/g) LSD=0.449	K^+ (mg/g) LSD=0.076	Ca^+ (mg/g) LSD=0.135
Aerva javanica(S)	1.532±0.048 (b)	1.098±0.027 (c)	4.403±0.029 (e)
Aerva javanica(L)	1.748±0.048(b)	1.120±0.053(c)	4.934±0.050(d)
	(-14.099)	(-2.004)	(-12.059)
Panicum antidotale(S)	1.907±0.255(ab)	1.127±0.047(c)	5.707±0.024(b)
	(-24.478)	(-2.641)	(-29.616)
Panicum antidotale(L)	2.234±0.044(a)	2.820±0.049(b)	5.318±0.039(c)
	(-45.822)	(-156.830)	(-20.781)
Panicum antidotale(R)	1.728±0.546(b)	5.013±0.021(a)	6.159±0.014 (a)
	(-12.794)	(-356.557)	(-39.882)

Means showing different letters differed significantly p ≤ 0.05, LSD= Least significant difference, S= Stem, L= Leaf, R= Root

Table 4. Mean comparison of phosphorus, potassium and and calcium contents (mg/g) in trees of Thal desert

Plant	Phosphorous (mg/g) LSD=0.075	K^+ (mg/g) LSD=0.069	Ca^+ (mg/g) LSD=0.066
Tamarix aphylla(S)	0.846±0.045 (b)	2.492±0.029 (b)	2.529±0.043 (b)
Tamarix aphylla(L)	0.929±0.030 (a)	4.396±0.043 (a)	2.729±0.034 (a)
	(-9.810)	(-76.404)	(-7.908)
Acacia modesta(S)	0.415±0.035 (c)	1.117±0.029 (c)	2.309±0.017 (c)
	(50.946)	(55.176)	(8.699)

Means showing different letters differed significantly p ≤ 0.05, LSD= Least significant difference, S= Stem, L= Leaf, R= Root

comparison showed significant differences among *Tamarix aphylla* stem (2.492 mg/g), *Tamarix aphylla* leaves (4.396 mg/g) and *Acacia modesta* stem (1.117 mg/g). *Tamarix aphylla* leaves showed maximum Calcium ion contents (2.729 mg/g) and *Tamarix aphylla* stem revealed minimum (2.529 mg/g) calcium contents. The comparison of means exhibited significant differences among *Tamarix aphylla* stem (2.529 mg/g), *Tamarix aphylla* leaves (4.729 mg/g) and *Acacia modesta* stem (2.309 mg/g).

4. DISCUSSION

The results showed that phytochemicals and nutrients were different in various species and their parts(Table 1-4). The differences 0f quantities are helpful in finding the importance of plant by many ways regarding the uilization of compound type present in plant. Due to the presence or absence of biochemicals like alkaloids, tannin, glycosides, coumarin, flavonoids and vitamins plants can be judged as being toxoc or medicinal [24]. The amount of phytochemicals and ions present in a plant determines the importance of plant as medicinal agent in addition to disclosing these as a new sources of economic materials [25,26]. These compounds are synthesized through primary and secondary metabolism. Of these compounds, a wide range are used in agriculture, veterinary and other scientific research [27]. Some metabolites also have been effective in role as inhibitory agents for microorganisms [28]. Antioxidants, polyphenols, vitamins and rcarotenoids are the active and effective role in avoiding oxidative stress related diseases [29,30]. Similarly ,antioxidant activity of plant is determined by the concentration of pheolics present in plant [31-33]. Tocopherols and β-carotene are also the potent factor for antioidant activity potential [34].The variations in concentration of phytochemicals and nutrients is species specific [35].

5. CONCLUSION

The concentration of metabolites and ions which determine the pharmacological potential of plants varied from species to species and also in various parts of the same species. In *Aerva javanica* stem the lowest alkaloid, tannin, soluble sugar, phosphorus, potassium and calcium contents were found. Among trees specimens, *Tamarix aphylla* leaves showed highest soluble sugar, phosphorous, potassium and calcium contents. The lowest alkaloid, terpenoid, phosphorous and potassium contents were found in stm of *Acacia modesta*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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