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The Anthelmintic and Antioxidant Activities of South African *Geranium Incanum*

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Abstract: *In screening several plant species from an inventory of common medicinal plants from South Africa for diverse medicinal purposes, Geranium incanum plant was selected for investigation because of its interesting ethnomedicinal profile. The present study was designed to evaluate the anthelmintic and antioxidant potentials in the different extracts and Isolates of the South African G. incanum. The anthelmintic activity was carried out using larval stage paralysis method and levamisole as reference drug. The larvae stage of Haemonchus contortus from faecal samples of infected rabbits that was obtained from cultured specimen was used for the analysis. The different extracts showed different level of inducement with the methanolic extract, induced approximately 85% larval paralysis within 24 h of contact with the H. contortus larvae at 20 mg/ml. This level of activity may be useful in animal husbandry practice to encourage animals to develop immunity against subsequent worm infections. This action gives a good ground for the use of the plant for treatment of helminthiasis in African traditional medicine. The total antioxidant assay also reveal a huge amount of excellent information into the nature and properties of each extracts and isolates in which the distribution of polyphenols, flavonoids and anthocyanins present in the plant extracts were revealed. The ORAC, FRAP and TEAC showed intensively the antioxidant capacity of the extracts and isolates in which it was evident that the methanolic extracts and isolates obtained from them are good antioxidant agents for which **G10** is even higher than the standard used in some case as shown in the results obtained. The total phenol varied from 14.1 ± 1 to 298.5 ± 5 mg/g in the extracts. Flavonoid contents were between 22.15 ± 0.8 and 81.3 ± 5.4 mg/g. The methanolic extract has higher ORAC and phenolic contents as compared to other extracts. The highest radical scavenging effect was observed in the methanolic extract with IC50 = 0.014 mg/ml. The potency of radical scavenging effect of the methanolic extract was about 5 times greater than a synthetic antioxidant butylated hydroxy toluene (BHT). Due to these 2 observed properties, the extracts of the plant might be used as a new multifaceted drug. The data obtained is a promising profile for development of a good anthelmintic and antioxidant agents for the future, especially compounds **G3** and **G10** as a single dose for both anthelmintic and antioxidant therapy.*

Keywords: *Geranium incanum; Haemonchus contortus; Larval paralysis; antioxidant; radical scavenging; Phenolic compound.*

1. INTRODUCTION

Medicinal plants have been used in many forms over the years to cure, manage or control man's ailments. Any effort to further maximize the output of medicinal or natural products from the botanical florals and so to improve health-care delivery certainly deserves great attention. This search led to the great wealthy and potential content of *Geranium incanium* a well known South African indigenous plant.

Geranium incanum is from the family Geraniaceae, it is refered to as Vrouebossie – amarabossie (Afrikaans) and ngope-sethsoha, tlako (Sotho). This plant is commonly found along the southern coastal areas of the Western and Eastern Cape Provinces of South Africa (Hilliard and Burtt, 1985). It is an attractive, sprawling perennial shrublet (Hilliard and Burtt, 1985). The leaves have been used as a tea substitute (Rood, 1994; Watt and Breyer-Brandwijk, 1962) for treating colic, diarrhoea, fever, bronchitis, bladder infections, venereal diseases and menstruation-related ailment, hence the common name vrouebossie ("vroue" = women; "bossie" = small bush) (Smith, 1966; Watt and Breyer-Brandwijk, 1962). It is very interesting to note that other *Geranium* species such as *G. robertianum* (Robert Herb) are traditionally used in Europe and America to treat diarrhoea (Amabeoku, 2009; Grieve, 1967). The leaves of *Geranium* species are known to contain flavonoids and tannins, of which geraniin is the best known compound and the indication common to all tannin-containing drugs is the symptomatic treatment of diarrhoea (Dic. Nat. prod., 2006; Amabeoku, 2009). Due to the use of *G. incanum* for the treatment of diarrhea and the noticeable increasing problems of development of resistance in helminths (Geert and Dorny, 1995; Coles, 1997) against anthelmintics agents have led to the proposal of screening this medicinal plant for its anthelmintic activity. A number of medicinal plants have been used to treat parasitic infections in man and animals (Said, 1969; Akhtar et al., 2000). The anthelmintic activity of *Geranium incanum* was chosen because of its effect as anti diarrhea. The commonest nematodes is Haemonchus *conturtos*

G. incanum is widely used in ethno-medicine system of South Africa for curing so many ailment. However, anthelmintic activity of this plant has not so far been scientifically proved. The present study was, therefore, carried out to assess the anthelmintic and antioxidant activities of *G. incanum.*

2. MATERIALS AND METHODS

2.1. Plant Materials Collection

The *Geranium incanum* were collected from an open field in Belhar area in Cape Town, one collection was made during summer in early march 2007 and another collection was made during winter in late August 2007. The plants have been authenticated by a taxonomist in the Department of Biodiversity and Conservation Biology. A voucher specimen prepared was deposited at the University Herbarium with voucher numbers: - Weitz 1013(UWC) for *G. incanum*.

2.2. Plants Preparation

The method previously described by Babajide et al., (2008) was used in which about 1kg each of the summer and winter collection were washed with distilled water separately and dried at room temperature in a ventilated room, milled to a fine powder and stored in closed containers in the dark in a deep freezer until use.

2.3. Sequential Extraction

The plant materials were sequentially extracted using the method described by Babajide et al., (2010) where each solvent extraction were made three times each for 24 hours respectively by continuous stirring with a mechanical stirrer, with n-Hexane(n-Hex), Dichloromethane(DCM), Ethyl acetate(EtOAc), Methanol (MeOH) and Water (H_2O). The combined solvents were separately evaporated under reduced pressure at 40°C using a rotavapor, while the aqueous extracts were concentrated by freeze-drying. All dried extracts were stored at -10^oC.

The extracts obtained is as follows: **GISH** = *G. incanum* summer collection Hexane extract; **GISD** = *G. incanum* summer collection DCM extract; **GISE** = *G. incanum* summer collection EtOAc extract; **GISM** = *G. incanum* summer collection MeOH extract; **GISW** = *G. incanum* summer collection H_2O extract; **GIWH** = *G. incanum* winter collection Hexane extract; **GIWD** = *G. incanum* winter collection DCM extract; **GIWE** = *G. incanum* winter collection EtOAc extract; **GIWM** = *G. incanum* winter collection MeOH extract; **GIWW** = *G. incanum* winter collection H_2O extract.

The isolation was carried out as shown in Babajide J.O., 2009 with a total of 12 compounds viz., (**G1** – **G12**), while only **G5**, **G10** and **G12** were fully analysed and reported. The isolation and characterization were carried out using similar method as shown in Babajide et al., 2015.

3. IN VITRO ANTHELMINTIC ACTIVITY

The anthelmintic activity was also carried out using the in vitro method.

The in vitro trials for anthelmintic activity of the extracts, and the isolates were conducted on mature live *Haemonchus contortus* of infected rabbits as described previously by Sharma et al., 1971. Briefly, the mature worms were collected from the abomasums of freshly slaughtered rabbits. The worms were washed and finally suspended in phosphate buffer saline (PBS). Ten worms were exposed in triplicate to each of the following treatments in separate Petri dishes at room temperature (25–30 ◦C):

- Levamisole 0.55 $mgml^{-1}$.
- Different extracts of *G. incanum* at 20 $mgml^{-1}$ (different extracts).
- PBS.

The inhibition of motility of the worms kept in the above treatments was used as the criterion for anthelmintic activity. The motility was observed on 0, 1, 2, 3 6, 12 and 24h intervals. Finally, the treated worms were kept for 30 min in the lukewarm fresh PBS to observe the revival of motility.

4. ANTIOXIDANT ASSAY

Total antioxidant evaluation was carried out on all the extracts, fractions and isolates by first evaluating (a) Antioxidant content of the following:-

- Polyphenols (Garllic acid)
- Flavonols (Quercetin)
- Flavanols (Catechin)
- Anthocyanins
- Flavanones (Naringenin)

(b) Antioxidant Capacity for:-

- ORAC (Oxygen radical absorption capacity) by H^+ ion transfer.
- FRAP (Ferric reducing antioxidant Power) by the movement of e^- electron.
- TEAC (Trolox (a well known standard) equivalent antioxidant capacity)

All readings were taken after 40 minutes unless otherwise stated and the Microsoft Excel workbook program was used in all the analysis and calculations.

The evaluation of Polyphenolics contents was carried out using the Folin Ciocalteu reagent with garllic acid as the standard to measure the total polyphenols in the samples as described in Babajide J.O., 2009 while evaluation of Flavonols contents make use of quercetin as the standard for evaluating the amount of flavonols in the sample at 360nm.

The Flavanols content makes use of 4-dimethylaminocinnamaldehyde (DMACA) which reacts with flavanols to form a characteristic light blue colour that can only be measured at 640nm with catechin as standard. Anthocyanins measurement was made using total monomeric anthocyanin measurement by the ph-differential method as described in Babajide J.O., 2009. Evaluation of Flavanones makes use of Naringenin as the standard for measuring total Flavanones in the sample using 2, 4-dinitrophenylhydrazine (DNPH).

The second stage is the OXYGEN RADICAL ABSORBANCE CAPACITY ASSAY (ORAC)

The ORAC method was performed using a fluorescence spectrophotometer while Ferric Reducing Antioxidant Power Assay (FRAP) assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant present in stoichiometric excess. The last method is the Abts Trolox Equivalent Antioxidant Capacity (TEAC) assay which uses the same method as ORAC.

5. RESULTS AND DISCUSSION

The whole plant of *G. incanum* was collected at two different periods, one in winter (w) and the other in summer (s) because of the seasonal variation observed in the constituent of the plant.

The collections were made at two occasions at different times of the year due to insufficient amount of samples made at first collection and it was discovered that *G. incanum* collected in summer was partially phytochemically different from that collected during winter as shown in Babajide et al., 2010. The reason for this variation is suspected to be due to the higher nutrients absorption rates and water intake by the plant during winter which is more than that available during summer in South Africa (Clark et al., 2008; Naqinezhad et al., 2008).

The plant was washed, dried, milled separately and sequentially extracted as shown in Babajide et al., 2010 which gave **GISH**, **GISD**, **GISE**, **GISM, GISW**, **GIWH**, **GIWD**, **GIWE, GIWM** and **GIWW** respectively as shown in section 2.3 above.

The Phytochemical screening for the detection of natural plant products in the extracts were targeted for only tannins, phenolics, glycosides, saponins, flavonoids, alkaloids, anthraquinones and essential oils which were performed according to the method of Wagner (Wagner and Bladt, 2001).

The profile showed that the secondary plant metabolites present in the different extracts are diverse which equally affects the brine shrimp cytotoxicity and the antimicrobial profile as shown in Babajide et al., 2010.

A total of 12 compounds were successfully isolated from both the summer and winter collections of the plant material because of its seasonal variation viz., (**G1** – **G12**), with only **G2, G3, G5**, **G10** and **G12** being fully analysed and reported while the remainder cannot be conclusively identified due to insufficient amount as at the time of this report. **G2** is a Geranine while **G3** is given the trival name Geranin, **G5** *Quercetin*, **G10** *16α-Hydroxy-(-) kauran-18-oic acid*, and **G12** *6, 8-di-C-methylquercetin 3, 7-dimethyl ether*. Four (4) of the characterized compounds were believe to be novel, the Geranine, Geranin, kauran-18-oic acid, and C-methylquercetin 3, 7-dimethyl ether (Babajide J.O., 2009).

5.1. In Vitro Anthelmintic Activity

The methanolic extract of both the summer and winter collections exhibited high anthelmintic activity against *Haemonchus contortus* as evident from the mortality of the worms (Table 1). The activities in that of water is low as shown in the result in which paralysis of *Haemonchus contortus* were observed in their motility, however, was revived after they were placed in PBS for 30 min (Table 1). All the worms exposed to 0.55mg/ml of levamisole were found dead at 6 h and at 1.00mg/ml of G3, G10 and G12 showed similar trend as found in the levamisole in which G10 was even stronger at the concentration used (Table 1); whereas, none of the worms were found dead or paralyzed in PBS. These findings suggested the presence of varying degrees of anthelmintic activity in the different extracts of *G. incanum*. For in vitro studies, *Haemonchus contortus* proved to be a good test worm because of its longer survival in PBS. This worm and some other *Strongyloides* have previously been used for in vitro studies by some workers (e.g., Sharma et al., 1971; Prakash et al., 1980; Amorium et al., 1998; Asuzu and Njoku, 1996; Sangwan and Sangwan, 1998).

Table1. *In vitro effect of Geranium incanum extracts and isolates on Haemonchus contortus of infected rabbits in comparison with positive control (levamisole)*

Treatment	Means number of worms showing motility at different hours							
	0h	1h	2h	3h	6h	12h	24h	Fresh PBS/30mins
Levamisole at 0.55 mg/ml	10.0a	3.6b	1.6c	0.3d	0d	0d	0d	0d
GISH at 20mg/ml	10.0a	10.0a	8.3b	7.0c	6.4d	5.2e	5.2e	7.0e
GISD at 20mg/ml	10.0a	10.0a	8.5b	8.1c	7.0d	5.1e	5.0e	6.5e
GISE at 20mg/ml	10.0a	7.8b	6.1c	4.2d	2.4e	2.0e	2.0e	2.0e
GISM at 20mg/ml	10.0a	10.0a	2.4b	0d	0d	0d	0d	0d
GISW at 20mg/ml	10.0a	10.0a	10.0a	9.3b	7.0c	5.6d	3.8e	4.2e
GIWH at 20mg/ml	10.0a	10.0a	8.6b	7.4c	6.9d	5.5e	5.5e	7.2e
GIWD at 20mg/ml	10.0a	10.0a	8.5b	8.1c	7.0d	5.1e	5.0e	6.5e
GIWE at 20mg/ml	10.0a	7.8b	6.1c	4.2d	2.4e	2.0e	2.0e	2.0e
GIWM at 20mg/ml	10.0a	4.7b	2.3c	1.2d	0.6e	0e	0e	0e
GIWW at 20mg/ml	10.0a	10.0a	10.0a	9.7b	7.3c	5.8d	3.9e	4.6e
G3 at 1.00mg/ml	10.0a	4.1b	2.1c	0.8d	0e	0e	0e	0e
G10 at 1.00mg/ml	10.0a	2.9b	1.1c	0d	0d	0d	0d	0d
G12 at 1.00mg/ml	10.0a	10.0a	3.8b	2.3c	0.6d	0e	0e	0e
PBS	10.0a	10.0a	10.0a	10.0a	9.6a	9.6a	9.6a	9.6a

a–e, means marked with the same letter in a row do not different significantly at P ≥ 0.05.

a Indicates that worms were placed in PBS after exposure of 6 h to the treatments to confirm their mortality.

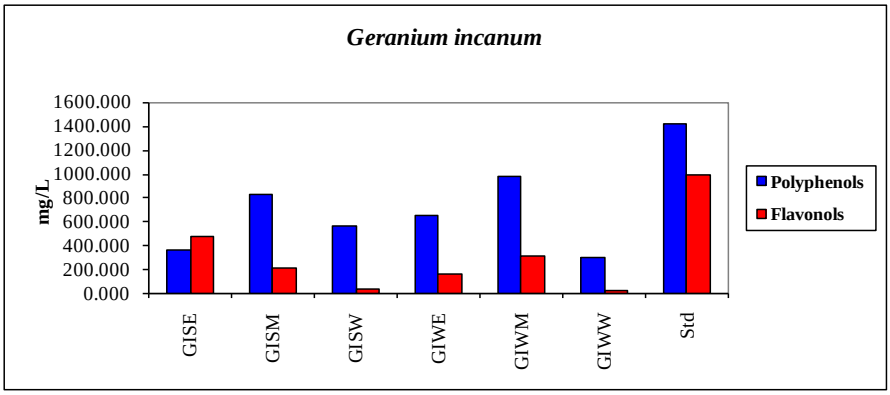
In vivo tests no doubt give more reliable data, but they require greater amount of compound, large number of animals and much time. The method described above is simple and economical. Worms from few animals are sufficient to test many drugs and their concentrations and only a little amount of chemical compound/plant extract is required. Moreover, no previous toxicity tests are necessary. The drugs which have been given by mouth reach the parasite in the intestine without much opportunity for chemical modification. This method can, therefore, be used for screening compound/plant extracts against intestinal worms. It is, however, true that no single chemotherapeutic test can be guaranteed to detect 100% of the active compounds/plant extracts. But as a compromise between time, expense and labor the test used in the current study is good.

5.2. The Antioxidant Assay

Total antioxidant evaluation were also carried out on *G. incanum* extracts and isolates by evaluating both the antioxidant content and the antioxidant capacity as shown above in section 3.2

5.2.1. Polyphenolics Content

The polyphenolic content was also evaluated as shown in section 3.2. The results showed that the amount of polyphenols present in the extracts varied quite a bit as observed in Figure 1A in which **GIWM** has the highest amount while the lowest was recorded for **GIWW** which presumably meant that the methanol extraction process removed a large portion of the polyphenols present.

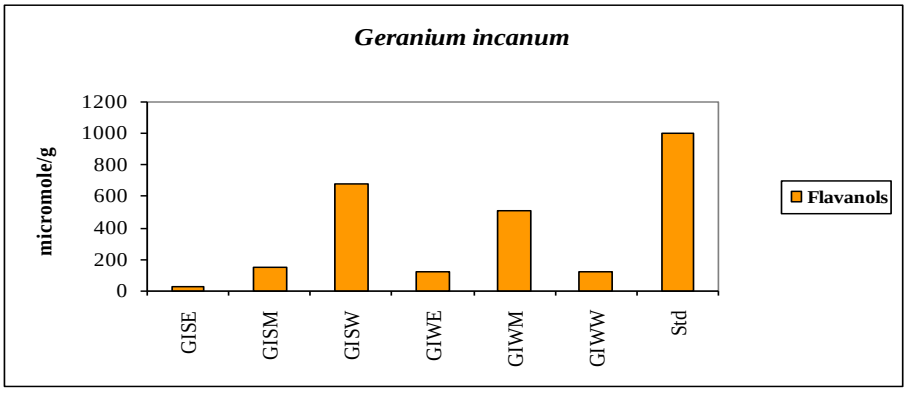


Extracts of *G. incanum*

Fig1A. *Bar Chart illustrating and comparing the amount of Polyphenols and Flavonols present in the various extracts of G. incanum.*

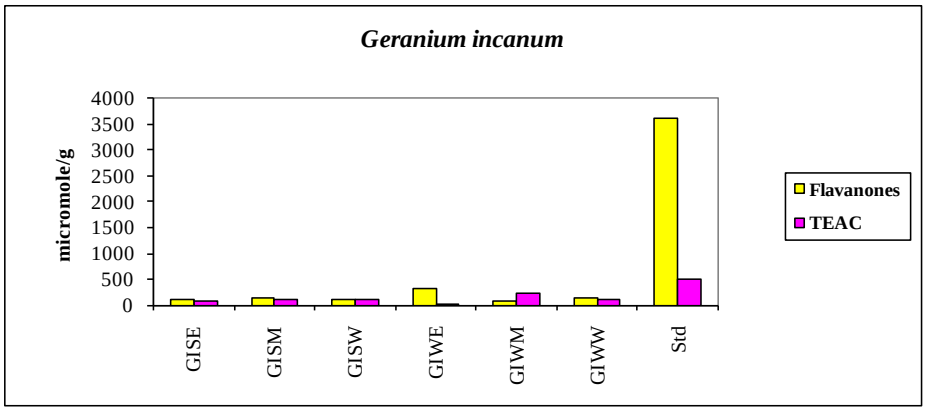
5.2.2. Flavonoids Contents

Flavonoids consist of flavonols, flavanols and flavanones which were also determined as described earlier and the results are shown in Figures 1A, 1B and 1C. From these it is evident that more flavonols were present in the ethyl acetate extract of the summer collection and the methanolic extract of the winter collection. The flavanol content is greater in the water extract of the summer than in winter while the methanolic extract of the winter plant recorded a significant presence of flavanols. Flavanones were found in trace amounts only as shown in Figure 1C.



Extracts of *G. incanum*

Fig1B. *Bar Chart showing the amount of Flavanols present in the various extracts of G. incanum.*

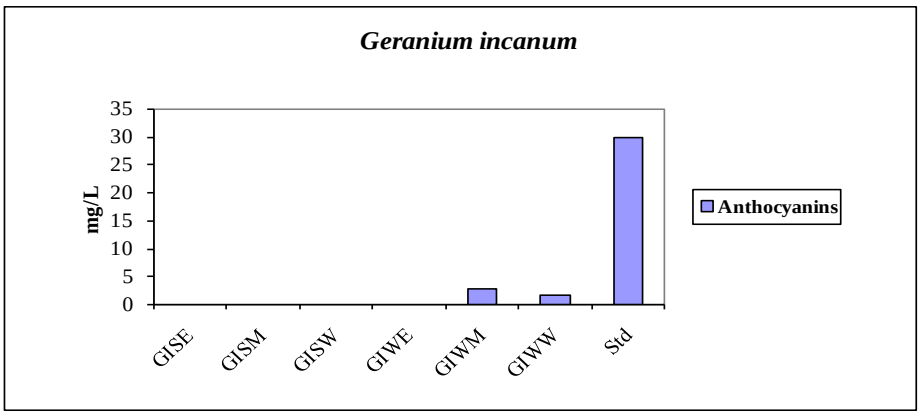


Extracts of *G. incanum*

Fig1C. *Bar Chart illustrating and comparing the amount of Flavanones and the Trolox equivalent antioxidant capacity present in the various extracts of G. incanum.*

5.2.3. Anthocyanin Contents

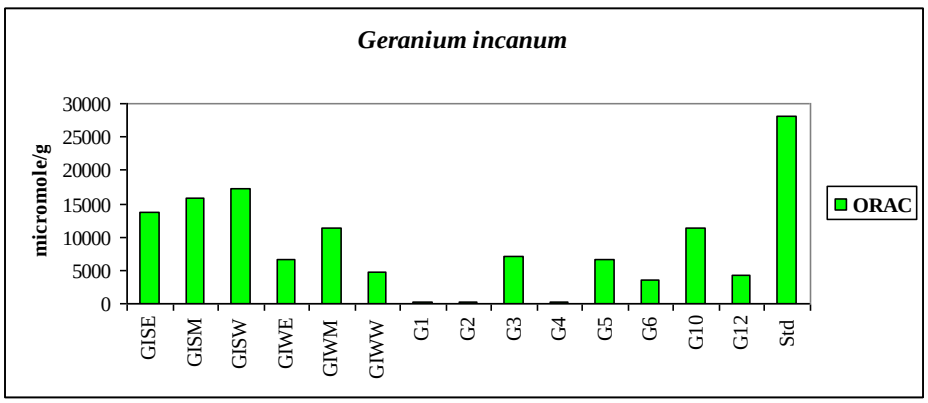
Results obtained (Figure 1D) clearly showed the absence of anthocyanins. Traces were only observed during winter in the methanol and water extracts.



Extracts of *G. incanum*

Fig1D. *Bar Chart showing the amount of Anthocyanins present in the various extracts of G. incanum.*

The **ORAC** (Figure 1E), **FRAP** (Figure 1F) and **TEAC** (Figure 1C) followed the same pattern, where the antioxidant capacity were fairly distributed except for compounds **G1**, **G2** and **G4** with very low ORAC values as shown in Figure 1E. The same pattern is found in the FRAP and TEAC evaluations as shown in Figures 1F and 1C respectively. There is a reasonable indication that the methanolic extract of the winter collection would be a strong antioxidant as shown by the high value in Figure 1F.



Extracts and isolates of *G. incanum*

Fig1E. *Bar Chart showing the ORAC values (The antioxidant capacity) of some extracts and isolates present in G. incanum.*

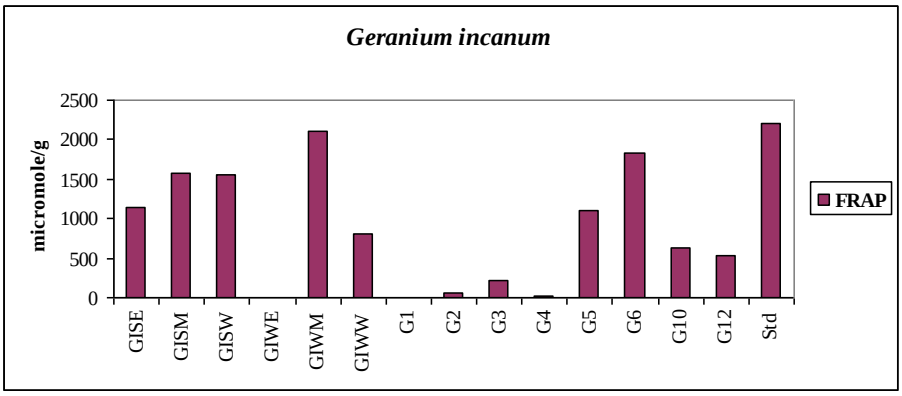


Fig1F. *Bar Chart showing the FRAP values (Ferric Reducing Antioxidant Power) of some extracts and isolates present in G. incanum.*

Generally, the total antioxidant assay reveal a huge amount of excellent information into the nature and properties of each extracts and isolates in which the distribution of polyphenols, flavonoids and anthocyanins present in the plant extracts were clearly shown. The ORAC, FRAP and TEAC showed intensively the antioxidant capacity of the extracts and isolates in which it was evident that the methanolic extracts and isolates obtained from them are good antioxidant agents in which **G10** is even higher than the standard used in some case as shown in the results obtained. The total phenol varied from 14.1 ± 1 to 298.5 ± 5 mg/g in the extracts. Flavonoid contents were between 22.15 ± 0.8 and 81.3 ± 5.4 mg/g. The methanolic extract has higher ORAC and phenolic contents as compared to other extracts. The highest radical scavenging effect was observed in the methanolic extract with IC50 = 0.014 mg/ml. The potency of radical scavenging effect of the methanolic extract was about 5 times greater than a synthetic antioxidant butylated hydroxy toluene (BHT). Due to these 2 observed properties, the extracts of the plant might be used as a new multifaceted drug. The data obtained is a promising profile for development of a good anthelmintic and antioxidant agents for the future, especially compounds **G3** and **G10** as a single dose for both anthelmintic and antioxidant therapy.

6. CONCLUSION

This study is a preliminary evaluation of the anthelmintic and antioxidant activities of the extracts and isolates in *G. incanum* and I strongly believe that further in-depth into the study of the extracts and isolates may generate novel anthelmintic, antioxidant, antimicrobial and antiviral metabolites. The extracts demonstrating anthelmintic activity could result in the discovery of novel anthelmintic agents while the extracts and isolates demonstrating broad spectra of activity, may help to discover new chemical classes of antioxidants and anthelmintics that could serve as selective agents for the maintenance of animal or human health hence providing biochemical tools for the study of infectious diseases.

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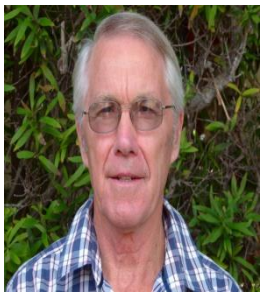

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