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Full Length Research Paper

Phytochemical screening and biological activity studies of five South African indigenous medicinal plants

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Different extracts and fractions of five selected indigenous South African medicinal plants, namely, *Cissampelos capensis*, *Geranium incanum* and three *Gethyllis* species, were subjected to phytochemical screening and testing for cytotoxicity using the brine shrimp lethality bioassay, and antimicrobial activity assays against nine microbes, which included three fungal species, three Gram negative and three Gram positive bacteria.The majority of the extracts tested positive for the presence of tannins, phenolics and flavonoids, while in selected cases, phytochemical tests suggested the presence of essential oils, glycosides or alkaloids. The methanol extract of *Gethyllis gregoriana* displayed the highest cytotoxicity levels. Generally, the highest levels of biological activity were shown to reside in the methanolic extracts, while hexane extracts revealed very low to zero activity. The total tertiary alkaloid (TTA) of *C. capensis* was mostly active against *Bacillus subtilis,* a Gram +ve bacteria. The trends observed for the cytotoxicity assay were in agreement with those observed for the antimicrobial assay.

Key words: Brine shrimp, lethality, medicinal plants, cytotoxicity, antimicrobial activity.

INTRODUCTION

Plants have long served as prolific sources of useful drugs, foods, additives, flavouring agents, lubricants, colouring agents and gums from time immemorial (Keay et al., 1964). The healing power of herbs had been recognized since ancient times and botanical medicine is one of the oldest practiced professions by mankind (Van Wyk and Gericke, 2000; Iwu, 1993). The search for new drugs to combat the problem of drug resistance, has in recent times, been receiving more attention (Coates et al., 2002; Henry, 2000) and hence recorded information, available from traditional medicine and ethnobotanical knowledge, has proved invaluable in this regard.

In South Africa, a large part of all medicine consumed each day is still derived from plants. Large volumes of plants or their extracts, packaged in different ways, are sold in both the informal and commercial sectors. According to national health experts, in South Africa (Buckingham, 1996), over 1000 different plant

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materials are used in medicinal preparations for either internal or external applications. On the other hand, the World Health Organization has compiled an inventory of more than 20,000 species of medicinal plants which are used for a variety of applications (Buckingham, 1996; Hoffmann et al., 1993). Some of these species have been tested for antimicrobial properties, while the majority are yet to be evaluated. Documentation of cytotoxicity and antimicrobial properties of medicinal plants is necessary in order to build a comprehensive database from which it may be possible to search for new leads in drug development when the need arises.

In recent times, many laboratories have been using bioassays to monitor the cytotoxicity of extracts and subfractions against the nauplii of *Artemisia salina*. The (LD_{50}) lethality of substances to brine shrimp nauplii has been linked to the probable ability of such compounds to kill cancer cells (antitumor activity), and possibly pesticidal and antimicrobial (antibacterial) activities. On the other hand, compounds that slow down the activity of the nauplii are usually expected to have an effect on the central nervous system (Mc Laughlin et al., 1991; Adesanya, 1994). Consequently, further tests often need to be performed on isolated active compounds as a means of evaluating the specific potential activities listed.

The aim of this study was to investigate the antimicrobial and cytotoxicity of these five selected indigenous South African plant species and to conduct their respective phytochemical screening. The selection of pathogenic organisms for the study was made based on their clinical and pharmaceutical importance to the health sector of the population.

The five plants selected are *Cissampelos capensis*, *Geranium incanum* and three *Gethyllis* species. These plants were selected on the basis of recorded ethnobotanical knowledge, evidence for their continued wide usage and local availability.

C. capensis is a member of the Menispermaceae family, which consists of 75 genera and 520 species (De Wet and Van Wyk, 2008).This plant is widely distributed in the sandy slopes and scrublands of the Northern, Western and Eastern Cape Provinces of South Africa and northwards into Namibia. Locally given vernacular names include “Dawidjiewortel” (Afrikaans) and “Mayisake” (isiXhosa). It is a perennial climber with twining stems and rounded, bright green leaves (VanWyk et al., 1997) without tendrils. It supports itself by twining around the stems of other plants, fences or walls (Smith, 1966). It is traditionally used to treat a variety of ailments such as gravel and glandular swelling, gallstones, dysentery, infections, menstrual problems, cholera, colic, bladder problems, snakebite, pains, toothache, measles, fever, diabetes, tubercu-losis, stomach and skin cancers, ulcers and syphilis sores. It is also used for the prevention of miscarriages, as an appetite stimulant and purgative, as well as for blood purification, (Van Wyk and Gericke, 2000; De Wet and Van Wyk, 2008; VanWyk et al., 1997; Smith, 1966; Rood, 1994; Watt and Breyer-Brandwijk, 1962; Steenkamp, 2003; Barbosa-Filho et al., 2000; De Wet et al., 2005). Several biologically active alkaloids of the bisbenzyltetrahydroisoqunoline type have been isolated from some *Cissampelos* species and other genera of the Menispermaceae (Buckingham, 2006, de Freitas et al., 1995). In particular, the two known aporphine alkaloids *viz*. (S)-dicentrine and (S)-neolitsine (Ayers et al., 2007) have been recently isolated from *C. capensis*. Some of these alkaloids have been implicated in sedative, antispasmodic and antitumour activities (Buckingham, 2006; VanWyk, 2008).

G. incanum is from the family Geraniaceae and 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. 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 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). The leaves of *Geranium* species are known to contain flavonoids and tannins, among which geraniin is the best known compound. The indication common to all tannin-containing drugs is the symptomatic treatment of diarrhoea (Buckingham, 2006).

Gethyllis species are from the family Amaryllidaceae and are refered to as Koekemakranka (Khoi, Afrikaans) or Kukumakranka (English). They are found only in southern Africa and grow abundantly in the Western and Northern Cape Provinces (Muller-Doblies, 1986; Elgorashi and Van Staden, 2004). All the plants in this group have an underground bulb with the scales thereof forming a distinctive neck at ground level. The long, thin leaves are usually spirally twisted, tattooed or coiled. The attractive flowers appear in summer when the leaves have already died. There are about 38 *Gethyllis* species, with *G. afra* and *G. spiralis* probably being the most abundant, (Muller-Doblies, 1986; Elgorashi and Van Staden, 2004) but newer species continue to be discovered and hence the list is increasing by the day. Koekemakranka brandy is one of the early Cape remedies for colic and indigestion (Smith, 1966; Rood, 1994; Watt and Breyer-Brandwijk, 1962; Forbes ed., 1986). The edible fruit was highly valued for its perfumery properties in rooms and on linen. Traditionally, an alcoholic infusion or tincture is made from ripe fruit containing some oils and esters of low molecular weight

alcohols.

MATERIALS AND METHODS

Plant materials collection

Whole plants of *Gethyllis multifolia* and *G. gregoriana* were obtained from Summerfield's Indigenous Bulbs and Seeds, Cape Town; they had been collected from Rawsonville and Vanrynsdorp respectively, while *G. villosa* was supplied by a horticulturist at the Cape Peninsula University of Technology in Cape Town. *Geranium incanum* was collected from an open field in the Belhar area near Cape Town, with one collection made during summer in early march 2007 while the other was made during the winter in late August 2007. *C. capensis* was collected from the University of the Western Cape Nature Reserve. All the plants were authenticated by a plant systematist, Mr F. Weitz, in the Department of Biodiversity and Conservation Biology. Voucher specimens were deposited at the University Herbarium with voucher numbers: Weitz 1013(UWC) for *G. incanum*; Weitz 1056(UWC) for *C. capensis*; Summerfield in UWC 6964(UWC) for *G. gregoriana*; Summerfield in UWC 6965(UWC) for *G. multifolia*; Summerfield in UWC 6966(UWC) for *G. villosa*.

Plant material preparation

Whole bulbs were used for the *Gethyllis* species (100 g each) and *G. incanum* (1 kg), while the aerial shoots (1 kg) and the roots (500 g) were separately processed for *C. capensis*. All the plant parts were washed with distilled water, dried at room temperature, milled to a fine powder and stored in closed containers in a deep freezer until ready for use.

Extraction

The plant materials were sequentially extracted with solvents of increasing polarity, with each extraction being repeated three times over a 24 h period by continuous stirring with a mechanical stirrer. The solvents used were n-hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH) and water (H_2O). The combined extracts from each solvent type were separately evaporated under reduced pressure at 40°C using a rotavapor, while the aqueous extracts were freeze-dried after concentration to a smaller volume. All dried extracts were stored at −10°C until further use.

The total tertiary alkaloid (TTA) (Barbosa-Filho et al., 1997) of *C. capensis* was obtained by macerating the dried powdered aerial shoot (500 g) in 80% EtOH at room temperature for 7 days. The dried ethanolic extract (100 g) was redissolved in 3% HCl and extracted several times with $CHCl_3$. The organic extracts were discarded while the aqueous fraction was basified with NH_4OH to pH 9 and again extracted with $CHCl_3$. The latter $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$) and the solvent evaporated to afford the TTA (1.85 g). The residual aqueous fraction was neutralized to pH 7 with 3% HCl and concentrated to dryness to afford a neutralized fraction called BTA (18.7 g). Only the methanol and water extracts were investigated for the *Gethyllis* species.

Phytochemical screening

Preliminary phytochemical screening of all the plant samples was carried out in order to determine the secondary plant metabolite profile of each (Wagner and Bladt, 2001).

Brine shrimp lethality bioassay

The brine shrimp lethality biossay was carried out using the following procedure:

Brine shrimp eggs (*Artemia salina* Leach) were hatched in sea water collected from the Cape Town seaside, using a large plastic case as an artificially partitioned dam. The eggs were incubated at room temperature for 48 h with the help of a light source and an aerator pump. The larvae (nauplii) were attracted to one side of the vessel where they were easily collected for the assay. All the plant extracts were dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration not exceeding 0.05% and then diluted with sea water for testing at the final concentrations of 10, 100 and 1000 µg/ml. Each test was conducted in triplicate. Ten nauplii were used for each test. Nauplii were counted under a magnifying glass after 24 h of incubation and maintaining the vials under illumination. The controls were prepared in the same way except that the test samples were omitted. The number of dead nauplii were recorded (lethality data) and it was used for calculating the LC_{50} at 95% confidence limit by the Finney Probit analysis program. LC_{50} values greater than 1000 ppm were considered inactive (Mc Laughlin et al., 1991; Babajide et al., 2008).

Determination of antimicrobial activity

The organisms used in the screening tests were obtained from both the National Collection of Type Cultures (NCTC) and American Type Culture Collection (ATCC). The Gram-negative bacteria were *P. aeruginosa* (NCTC 10332), *Proteus vulgaris* (NCTC 4175) and *Escherichia coli* Sero type 1 (NCTC 09001), while the Gram-positive were *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 13134) and *Bacillus licheniformis* (NCTC 01097). Fungal species used were *Candida albicans* (ATCC 90028), *Candida eropiralis* (ATCC 750) and *Aspergillus Niger* ATCC 10578. The fungi cultures were grown in a Sabouraud dextrose (SD) broth at 37°C and maintained on SD agar at 4°C.

A colony of each bacterial strain was suspended in 1 ml of Mueller–Hinton broth and incubated for 18 h at 37°C. After 6 h, a subculture was diluted 1/50 in the same broth before use. The disc diffusion assay was used to determine the inhibition of bacterial growth by the plant extracts (Rasoanaivo and Ratsimamanga-Urverg, 1993). Plant extracts were dissolved in the same solvents used for their extraction at a concentration of 100 mg/ml, and 20 µl were dispensed on a 9 mm sterile paper disc (Munktell/Lasec, Numb. FLAS3526009). Amoxicillin was used as a positive control (40 µg/ml) for bacteria while Fluconazole (120 µg/ml) was used for fungi. The diluted cultures were spread on sterile Muller–Hinton agar plates for the bacteria while SD was used for the fungi. The disc was placed at the center of each plate. The plates were then incubated at 37°C for 18–24h for bacterial pathogens and 3 days for fungal pathogens. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated. Antimicrobial activity was assessed by comparison of the inhibition zone of microbial growth (mm) produced by the plant extract to the inhibition zone around the Amoxicillin and Fluconazole standards (Vlietinck, 1997).

Table 1. Phytochemical screening results for the extracts.

Test material	Tann	Phen	Glyc	Sapo	Flav	Alka	Anth	Esse
GISH	+	+	-	-	-	-	-	+
GISD	+	+	-	+	+	-	-	+
GISE	+	+	-	+	+	-	-	-
GISM	+	+	-	+	+	-	-	-
GISW	+	+	-	+	+	-	-	-
GIWH	+	+	-	-	-	-	-	-
GIWD	+	+	-	-	-	-	-	-
GIWE	+	+	-	+	+	-	-	-
GIWM	+	+	-	+	+	-	-	-
GIWW	+	+	-	+	+	-	-	-
CCRH	-	+	-	-	+	-	-	-
CCRD	-	+	-	-	+	-	-	-
CCRE	+	+	-	+	+	+	-	-
CCRM	+	+	-	+	+	+		-
CCRW	+	+	-	+	+	+	-	-
CCAH	-	+	-	-	+	-	-	+
CCAD	-	+	-	-	+	-	-	+
CCAE	+	+	-	+	+	+	-	-
CCAM	+	+	-	+	+	+	-	-
CCAW	+	+	-	+	+	+	-	-
CCET	+	+	-	+	+	+	-	-
TTA	-	+	-	-	-	+	-	-
BTA	-	+	-	+	+	+	-	-
GGM	+	+	+	+	+	-	+	+
GGW	+	+	+	-	+	-	-	+
GMM	+	+	+	+	+	-	+	+
GMW	+	+	+	-	+	-	-	+
GVM	+	+	+	+	+	-	+	+
GVW	+	+	+	-	+	-	-	+

The key to the acronyms denoting different plant materials is as follows: [NB: numbers in brackets indicate the position of the letter in the acronym], First two blocks: G = Geranium, I = incanum, S = summer, ollection, H = Hexane extract, D = Dichoromethane extract, E = Ethyl acetate extract, M = Methanol extract, W (3) = Winter collection, W (4) = water extract , Third and fourth block: CC = *C. capensis*, R = root, H, D, E, M and W(4) = various extracts as in blocks 1 and 2 above, A = aerial shoot, Fifth block: Other aerial shoot extracts of *C. capensis*, CCET = 80% Ethanol extract; TTA = total tertiary alkaloid, RTA = neutralized aqueous residual fraction, Sixth block: G (1) = Gethyllis, G (2) = gregoriana M (3) = Methanol extract, W = water extract, M (2) = *multifolia*, V = villosa, Other column titles: Tann = Tannins, Phen = Phenolics,Glyc = Glycosides, Sapo = Saponins, Flav = Flavonoids, Alka = Alkaloids, Anth = Anthraquinones, Esse = Essential oils.

RESULTS AND DISCUSSION

The study of the five plant materials revealed diverse results for all the analyses performed (Table 1). Phytochemical screening for the detection of natural plant product classes present in the extracts was targeted only at tannins, phenolics, glycosides, saponins, flavonoids, alkaloids, anthraquinones and essential oils. The collection of *G. incanum* plant material was made on two separate occassions and at different times of the year due to insufficient quantities of material collected initially. It was thus discovered that the material collected in summer was phytochemically, partially different from that collected during winter (Table 1). In this regard, it was observed that the summer collection displays an extended positive distribution of saponins, flavonoids and essential oils, to include DCM extracts, which is not true for the winter collection. Furthermore, the hexane extract of the summer collection is rich in essential oils which were not detected in the corresponding extract of the winter collection. This variation may be linked to the stress associated with hot dry summers as opposed to the wet winters of the Western Cape region (Clark et al., 2009; Alireza et al., 2009). The secondary metabolite

profiles for the rest of the plant extracts are fairly diverse. It was discovered that anthraquinones were absent in most of the extracts except in the methanolic extracts of the *Gethyllis* species. Essential oils and glycosides were only found in the *Gethyllis* species while tannins, phenolics and flavonoids were readily detected in most of the extracts except in a few cases such as hexane and DCM extracts. Alkaloids tested negative for both the *G.incanum* and *Gethyllis* extracts, which is contrary to the observed trend in the Amaryllidaceae family where plants are generally known to be rich in alkaloids (Elgorashi and Van Staden, 2004). The presence of alkaloids was observed in *C. capensis* extracts, except the hexane and DCM fractions. These observations confirmed the phytochemical profile recorded in literature for some of these plant materials (De Wet and Van Wyk, 2008; Buckingham ed., 2006; VanWyk, 2008; Amabeoku, 2009).

Brine shrimp lethality assay

Bioactive compounds have been found to be toxic in high doses; hence the pharmacology of bioactive compounds can be preliminarily assessed from their toxicology results. The distribution pattern of bioactive plant metabolites in the extracts could be tentatively inferred from the brine shrimp cytotoxicity behaviour (Tables 2 and 3), where three bands of test results were designated as Inactive (LC_{50} > 700), Active (LC_{50} < 700) and Very active (LC_{50} < 10). The results showed that all the hexane extracts had no activity which may be an indication that none of the bioactivity resides in the hexane fractions. It is clearly evident that the highest bioactivity resides in the methanolic extracts of the plants. The high toxicity observed in the assay for *G. incanum* may be due to the presence of tannin, flavonoid and phenolic components which was demonstrated in the phytochemical screening results (Table 1). It has been reported in several studies that tannins have antimicrobial properties and high toxicity (Farthing, 2000; Powell and Field, 1980a). Bruneton reported that tannin-containing drugs are widely used for the treatment of microbial infections and hence the observed trend in *G. incanum* is in agreement with the results obtained (Powell and Field, 1980b).

The total tetiary alkaloid (TTA) fraction of *C. capensis* showed an LC_{50} value which reflects much higher activity *viz*. 0.3155 (Table 3) than the BTA with a value of 64.87. All the *Gethyllis* extracts were active except the water extract of *G. villosa*. The highest value in this experiment was recorded for *G. gregoriana* (0.223). It should be noted that *G. gregoriana* was active at all the concentration levels tested. This should be seen as an indication that it may serve as a good antimicrobial and antiviral agent, since the lethality of a test substance to brine shrimp nauplii has been linked not only to the possible ability of such substance to kill cancer cells (antitumor activity) but also to pesticidal and antibacterial activities. It may thus be deduced that all the “very active” samples should be good candidates for such applications. It is worthy to note that this is the first time *G. gregoriana* is the subject of a report for any bioassay studies, and to the best of our knowledge, this is the first time that cytotoxicity results based on the brine shrimp bioassay are being reported on these five plant species.

Antimicrobial activity evaluations

The extracts examined using the brine shrimp lethality bioassaywere subjected to antimicrobial evaluations. The results which were generated through observation of inhibition zones of microbial growth (mm) relative to that of a suitable reference compound are presented in Table 4. Similar patterns were observed in the microbial analysis except in very few cases. Three test organisms per set of Gram positive bacteria (*P. aeruginosa*, *Proteus vulgaris* and *Escherichia coli*), Gram negative bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Bacillus licheniformis*) and fungi (*Candida albicans, Candida eropiralis* and *Aspergillus niger*) were used in order to obtain a reasonably wide spectrum of antimicrobial activities of the extracts. *S. aureus*, a pyrogenic bacterium known to play a significant role in invasive skin diseases, was selected while *C. albicans* was also chosen for this study since it causes serious systemic infections, including an opportunistic infection in patients infected with HIV.

The results showed that 69% of all the extracts tested showed antimicrobial activity. Although the plant extracts were tested at concentrations about a thousand fold that of the standards, this needs to be seen in the context of the higher doses at which traditional medicines are generally administered compared to allopathic medicine. The highest activity was recorded against the Gram positive bacteria at 75% while that against Gram negative bacteria was at 68%. The largest zone (45 mm) of inhibition was observed for the TTA fraction of *C. capensis* against the Gram positive organism *B. subtilis.* This may be due to the strong antimicrobial and antiviral activities of the alkaloids in the plant; alkaloids are used for treatment of malaria, fungal infections, inflammation and cancer (Kaur et al., 2009; McGaw et al., 2000). No activities were recorded for the hexane extracts against all nine organisms. High values of activity were recorded even for the drug resistant breed of bacteria *viz. P. aeuruginosa*, where a zone of inhibition of 34 mm was recorded for GIWM, 41, 36, 25, 32 and 27 mm for GGM, TTA, CCAM, CCRM and GISM repectively. It is not an uncommon phenomenon for certain extracts to show preferential activity against selected organisms (Shai et

Table 2. The number of survivors counted after 24 h (Brine shrimp lethality test results).

Test materials	Vials at 1000 µg/ml			Vials at 100 µg/ml			Vials at 10 µg/ml			Vials for control expt.
	1	2	3	1	2	3	1	2	3	
GISH	6	8	4	9	7	10	10	10	10	10
GISD	10	8	8	8	9	10	10	10	10	10
GISE	2	3	3	5	5	6	8	6	8	10
GISM	0	0	0	1	2	2	4	5	4	10
GISW	1	2	2	4	4	5	9	6	8	10
GIWH	6	7	8	8	10	8	10	9	10	10
GIWD	2	2	1	4	4	6	7	9	7	10
GIWE	0	0	0	1	2	1	4	5	4	10
GIWM	0	0	0	0	0	0	1	2	1	10
GIWW	3	1	1	4	5	5	7	7	7	10
CCRH	9	9	10	10	10	10	10	10	10	10
CCRD	2	2	1	4	4	6	7	9	7	10
CCRE	2	3	3	5	5	6	7	6	8	10
CCRM	0	0	0	0	0	0	1	1	1	10
CCRW	2	3	2	4	4	4	6	7	8	10
CCAH	6	7	5	9	6	6	9	10	9	10
CCAD	2	1	2	5	4	4	7	6	7	10
CCAE	1	3	2	4	3	4	6	6	7	10
CCAM	0	0	0	0	1	0	2	3	2	10
CCAW	6	6	7	8	10	8	10	10	10	10
CCET	0	0	0	1	2	2	4	3	4	10
TTA	0	0	0	0	0	0	1	0	1	10
BTA	3	2	3	5	4	5	7	6	7	10
GGM	0	0	0	0	0	0	0	1	0	10
GGW	1	1	1	3	4	4	7	6	7	10
GMM	0	0	0	0	1	1	3	3	4	10
GMW	2	2	2	4	3	4	6	7	8	10
GVM	0	1	0	1	1	0	3	4	4	10
GVW	9	8	9	10	9	10	10	10	10	10

al., 2008) and this trend was observed in certain cases from the current study. Examples include GISD, which showed activity only against *B. lincheformis* and *C. albicans*. A similar observation was made for GISE, which is active against only 5 out of the 9 organisms; there was no activity against all the 3 fungi used. GIWD CCRD, CCRW and CCAE are other examples of this kind.

It is worthwhile to note that the literature indicates the majority of compounds found in the Amaryllidaceae family as alkaloids (Muller-Doblies, 1986; Elgorashi and Van Staden, 2004), yet none of the extracts from the *Gethyllis* species used, tested positive for alkaloids. Nevertheless, some of them were active against a few bacteria, probably due to the presence of flavonols, organic acids, glycosides and/or essential oils. Nothwithstanding the generally high cytotoxicity reported for members of the Amaryllidaceae family, some species continue to be administered orally as medicine or consumed as porridge by local people (Elgorashi and Van Staden, 2004).

In general, flavonoids and phenolics are known to exhibit a wide range of activities including anti inflammatory, antithrombotic, antiviral and hepatoprotection, which, in some measure, may be due to their ability to scavenge free-radicals (Mukhlesur et al., 2007; Ibewuike et al., 1997). A few specific flavonoids have been reported to be potent inhibitors of indole-3-acetic acid oxidase activity while some have been shown to exhibit strong lipid peroxidation inhibitory effects and cytotoxicity (Mukhlesur et al., 2007) when tested against oral micro-organisms. The present findings on the antimicrobial activities of the plant extracts which contain flavonoids and phenolics provide justification for the usage of

Table 3. The average number dead, counted after 24 h.

Test materials	Vials at 1000 µg/ml	Vials at 100 µg/ml	Vials at 10 µg/ml	LC_{50}	General remarks
GISH	4	1	0	1759.62	Inactive
GISD	1	1	0	989.66	Inactive
GISE	7	5	3	110.25	Active
GISM	10	8	6	6.18	Very active
GISW	8	6	2	88.35	Active
GIWH	3	1	0	4156.21	Inactive
GIWD	8	5	2	*	Active
GIWE	10	9	6	5.63	Very active
GIWM	10	10	9	0.4335	Very active
GIWW	8	5	3	76.24	Active
CCRH	1	0	0	3526.22	Inactive
CCRD	8	5	2	120.48	Active
CCRE	7	5	3	71.33	Active
CCRM	10	10	9	3.75	Very active
CCRW	8	6	3	100.03	Active
CCAH	4	3	1	*	Inactive
CCAD	8	6	3	145.86	Active
CCAE	8	6	4	58.64	Active
CCAM	10	10	8	6.1523	Very active
CCAW	4	1	0	2446.33	Inactive
CCET	10	8	6	*	Very active
TTA	10	10	9	0.3155	Very active
BTA	7	5	3	64.87	Active
GGM	10	10	10	0.2229	Very active
GGW	9	6	3	120.66	Active
GMM	10	9	7	6.201	Very active
GMW	8	6	3	139.55	Active
GVM	10	9	6	4.233	Very active
GVW	1	0	0	1956.39	Inactive

*Data did not converge and therefore could not be regressed by the finnery probit analysis programme.

Table 4. Antimicrobial activity profile of the plant extracts.

	Psa	Prv	Esc	Bas	Sta	Bal	Caa	Cae	Asn
Amx	61	44	49	42	53	47	0	0	0
Flu	0	0	0	0	0	0	46	53	39
GISH	0	0	0	0	0	0	0	0	0
GISD	0	0	0	0	0	12	22	0	0
GISE	19	24	18	26	0	28	0	0	0
GISM	27	32	32	38	29	33	30	23	27
GISW	0	0	12	15	13	0	18	21	15
GIWH	0	0	0	0	0	0	0	0	0
GIWD	0	0	0	19	15	22	26	20	23
GIWE	29	26	35	38	30	18	15	17	22
GIWM	34	41	28	36	36	31	30	27	29
GIWW	10	11	15	0	0	20	22	23	28
CCRH	0	0	0	0	0	0	0	0	0
CCRD	0	12	11	13	18	15	0	0	0
CCRE	14	0	15	17	33	19	17	14	20
CCRM	32	28	28	43	35	27	30	36	23
CCRW	0	0	0	11	17	12	25	26	25
CCAH	0	0	0	0	0	0	0	0	0

Table 4. Contd.

CCAD	15	18	16	11	13	11	0	0	0
CCAE	14	12	12	17	18	0	0	0	0
CCAM	25	21	20	35	40	44	23	22	19
CCAW	11	12	11	10	24	11	17	15	29
CCET	24	22	31	37	35	28	36	33	34
TTA	36	38	37	45	40	36	38	33	35
BTA	15	17	17	23	28	24	30	32	29
GGM	41	34	29	32	30	33	27	29	23
GGW	16	19	19	22	19	14	19	15	17
GMM	18	21	26	19	18	18	10	11	11
GMW	10	13	11	12	10	11	0	0	14
GVM	15	13	19	22	28	30	15	17	17
GVW	0	0	0	0	0	0	0	0	0

Amx (Amoxicillin), Flu (Fluconazole), Gram –ve bacteria: - *P. aeruginosa* (Psa), *Proteus vulgaris* (Prv) and *Escherichia coli* (Esc), Gram +ve bacteria: - *Bacillus subtilis* (Bas), *Staphylococcus aureus* (Sta) and *Bacillus licheniformis* (Bal), Fungi: - *Candida albicans* (Caa)*, Candida eropiralis* (Cae) and *Aspergillus niger* (Asn).

these plants in folk medicine for the treatment of viral infections, oral sores and inflammations as described in their ethnomedicinal uses (Liu et al., 1990).

Conclusion

This preliminary investigation of these plants has clearly demonstrated their cytotoxic, antimicrobial and antiviral properties. Further indepth studies may lead to the isolation of compounds with a potential to be used as drugs in themselves or as templates for novel drug development. The extracts having anticandidal activity could result in the discovery of novel anticandidal agents, while the plants demonstrating a broad spectrum of activity, may serve as leads to discover new chemical classes of antibiotics, that could serve as selective agents for the maintenance of animal or human health and also provide biochemical tools for the study of infectious diseases.

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