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Phytochemical Analysis and Screening of Ugandan Medicinal Plants for Antifungal Activity against *Candida albicans*

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Authors' contributions

The research was carried out in close collaboration between all the authors. Author AF under the guidance of authors KE and OOJ designed the study and carried out laboratory experiments. Authors KE and AG wrote the first draft of manuscript, managed literature searches and data analysis. All authors have all read and approved the final version of the manuscript.

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ABSTRACT

Aims: The potential activity against *Candida albicans* of five commonly used medicinal plant species of Bwindi Impenetrable Forest National Park in southwestern Uganda was investigated.
Study Design: The phytochemical profiles of *Tetradenia riparia, Erucastrum arabicum, Plectranthus lactiflorus, Solanecio mannii* and *Platostoma africanum* were analysed.
Place and Duration of Study: The experiments were carried out in the Department of Pharmacology and Therapeutics, College of Health Sciences, Makerere University and Department of Microbiology and Parasitology, College of Veterinary Medicine and Biosecurity, Makerere University, between September 2012 and January 2013.

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Methodology: The Agar well diffusion method was used to measure the antifungal activity against *Candida albicans* (ATCC 10231). The Minimum Inhibitory Concentration (MIC) was determined by the serial dilution method and the phytochemical analyses were made by standard of the plant extracts phytochemical tests.
Results: Antifungal activity of both ethanol and diethylether extracts of *Tetradenia riparia* surpassed the Nystatine standard (31 and 28 > 25, respectively) as well as those of other four plant species. The MIC values for the ethanol and ether crude extracts were 0.0312 and 0.0156 g/ml respectively. The plant species tested proved to be positive for different phytochemicals including tannins, coumarins and steroidal glycosides.
Conclusion: Generally, all plant species proved to be active against *Candida albicans. Tetradenia riparia* exhibited the highest antifungal activity and it is considered to be a potential source of antifungal drugs.

Keywords: Fungal infections; herbal medicine; Bwindi; Candida albicans; medicinal plants; Tetradenia riparia.

1. INTRODUCTION

The frequencies of fungal infections are recently increasing [1]. Pathogenic fungi in general and *Candida albicans* in particular, are more difficult to control than non-pathogenic fungi [2-4]. *C. albicans* remains the most frequent cause of fungal infections in an expanding population of immuno-compromised patients [2], and also plays a role in sexual transmitted infections [5]. The pathogenic fungi produce superficial infections, not only in immune-compromised hosts but also in healthy individuals, living under poor sanitary conditions, especially in the developing world [6]. Candidiasis has become a major public health problem as an opportunistic infection of HIV/AIDS [7]. Bacterial and fungal infections have been reported to be prevalent in Uganda [8]. Fungi widely affect different body parts like skin, hair and nails [9].

There are several challenges that affect the successful treatment of fungal infections. These include limited and costly drugs, microbial drug resistance, toxicity, and relapse of infections [10,11]. The search for novel antifungal agents relies in great part on ethnobotanical information and ethnopharmacologic exploration [12,4]. Plants have been found to play a major role in managing dermatological conditions [13,14]. Svetaz et al. [15] found a significantly higher probability ($p<0.01$) of detecting plants with antifungal activity (MIC≤1000 µg/mL) against at least one fungus when plants possess reported ethnopharmacological uses related to fungal infections. Therefore medicinal plant species have a great potential as good sources for new and safe antifungal drugs [3].

Kamatenesi et al. [8] reported that people in rural parts of Uganda use herbal drugs to treat bacterial and fungal infections. In Bwindi, a typical rural area in southwestern Uganda, the people do not have access to modern medical facilities and services. They, highly depend on traditional medicinal herbs to treat many ailments including fungal infections. The signs or symptoms that give support to a traditional antifungal use are mainly related to skin or mucosal conditions [15]. This study was therefore carried out to investigate the use of selected medicinal plant species used by this community and their potential in treating fungal skin infections.

2. MATERIALS AND METHODS

2.1 Study Area

Bwindi is situated in Kigezi highlands, southwestern Uganda, between 29º 35' to 29º 50' E and 0º 53' to 1º 8' S [16]. The altitude ranges between 1,190 m and 2,607 m with a rough terrain characterized by numerous steep sided hills and narrow valleys of a general incline from the south western to the northwestern parts. Geologically, the area is associated with up warping of the Western Rift Valley. The climate is tropical with two rainfall peaks from March to May and September to November. Annual rainfall intensity ranges between of 1,400 mm and 1,900 mm, while the temperature ranges between 7ºC and 15ºC [17].

2.2 Selection of Antifungal Medicinal Plants for Bioassay and Phyto-chemical Screening

Five of the most frequently used medicinal plant species in areas around BINP were selected for analysis of antifungal activity *in vitro.* The selection of plants for analysis was based on

their frequency of mention and preferred use by the traditional healers and the respondents interviewed. The 5 plant species selected were; *Tetradenia riparia* (Hochst.) Codd*, Erucastrum arabicum* Fisch & C.A. Mey.*, Plectranthus lactiflorus* (Vatke) Angew, *Solanecio mannii* (Hook. f.) C. Jeffrey and *Platostoma africanum* P. Beauv (Table 1).

2.3 Collection and Drying of Plant Materials

Voucher specimens of the plant species the herbalists use to prepare antifungal remedies were collected and taken for identification at the Makerere University Herbarium following the procedures described in [18]. The plant species were classified using Martin the Kew database at www.theplantlist.org accessed on 25/10/2014. In order to collect representative samples of plant specimens for phytochemical and antifungal analyses, procedures described in Hue et al*.,* [19] were followed. The plant materials were collected from the parishes of Rutugunda, Nteko, Rubuguri, Nyamabare, Mpungu, Mukono and Rubimbwa adjacent to the forest. They were air-dried at room temperature for 2 weeks to avoid decomposition of volatile chemical in plant materials. The different dried materials were separately pounded using mortar and pestle then sieved into fine powder, and stored in airtight jars.

2.4 Preparation of Extracts

Diethylether and ethanol were used as the extracting solvents for the plant materials. For each plant species, 35 g of powder was soaked in petroleum ether in series for 36 hours, with periodic shaking. The extract was filtered off and fresh solvent added. This was repeated 3-5 times until there was almost no more coloration acquired by the solvent. Each filtrate was then separately concentrated using a rotary evaporator. The dry residue was then further extracted using ethanol in series of 4 to 5 times as above (Buchi Rotavapor R-210) but as the solvent. After recovering the extracting solvents, the concentrated crude extracts were kept in separate containers and labeled appropriately.

2.5 Phytochemical Analysis of Ethanol Crude Extracts

Phytochemical screening of crude extracts was conducted to identify the major chemical groups in the extracts using standard tests as described by various authors [20,21].

2.6 Antifungal Susceptibility Tests

The Agar well diffusion assay was used to test the activity of each crude extract on a culture of *Candida albicans* (ATCC 10231) obtained from Department of Microbiology and Parasitology, College of Veterinary Medicine, Animal Resources and Biosecurity at Makerere University. Nystatin was used as the positive control since it is a polyene-macrolide antifungal antibiotic that is widely available for the topical treatment of localized fungal infections and is known to be effective against a variety of fungal infections in humans [22,23].

Dehydrated Mueller Hinton agar (38 g) was suspended in 1 liter of water and mixed well. The contents were boiled with frequent agitation for one minute to enable the powder dissolve completely. The resultant medium was autoclaved at 121ºC for 25 minutes and then cooled in a water bath at 45 to 50ºC, before being introduced into the sterilized Petri dishes. A representative sample of the medium was incubated at 27 to 30ºC for 24 hours and examined to verify its sterility. Four wells of approximately 4 mm diameter were dug into the Petri dishes. The entire surface of the agar was inoculated with *C. albicans*. After inoculation, the plates were incubated at 27 to 30ºC for 24 hours to allow the inoculum establish fully. One gram of extract was dissolved in 2 ml of Dimethyl sulphoxide (DMSO) separately introduced into the wells in the culture and incubated at 30ºC for 24 hours. The diameters of the zones of inhibition around the wells were measured.

2.7 Minimum Inhibitory Concentration (MIC) of Extracts

MIC was defined as the lowest concentration of extract at which no fungal growth was observed after incubation. The MIC of each plant extract was determined by serial double dilution method using Brain Heart Infusion Broth (BHIB).

3. RESULTS

The commonly mentioned fungal diseases in Bwindi according to an earlier ethnobotanical study by Kakudidi et al. [24] were *Candidiasis, Tinea capitis, T. pedis, T. corpolis, T. versicolor, T. manuum, T. unguium, and T. cruris*.

3.1 Growth Inhibition Zone

The activity of both ethanol and diethylether extracts of *T. riparia* against *C. albicans* sarpassed Nystatin (Table 1). However, Nystatin had a greater diameter of the zone of inhibition than all the other plant extracts. Ethanol extract of *T. riparia* had the highest diameter (31 mm) of all plants screened. The extracts of other plants except the ether extract of *P. lactiflorus,* and the ethanol extracts of *P. africunum* and *S. manii*, were active against *C. albicans.*

3.2 Minimum Inhibition Concentration

Ethanol extract of *T. riparia* had the lowest MIC against *C. albicans* (0.0156 g/ml) of all plants (Table 2). This was followed by the ether extract of the same plant *T. riparia* (0.0312 g/ml). All extracts apart from the ether extract of *P. latiflorus* and *S. manii* were active against *C. albicans.* The DMSO had not effect on the growth of *C. albicans.*

3.3 Qualitative Phytochemical Analysis

Different plant parts of the different plant species were analysed (Table 3). Tannins were present in ethanol extracts of all the plant species analysed. Coumarin derivatives and steroid glycosides were also detected in all cases. None of the plant species analysed was positive for emodols. Anthocyanin pigments were only present in the hydrolysed ethanol extract of *T. riparia*. None of the plant species analysed was positive for alkaloid salts. Only *P. latiflorus, S. manii* and *P. africunum* contained basic alkaloids. Flavanosides were present only in the ether extract of the roots of *P. africunum* and the hydrolised ethanol leaf extract *of S. manii*.

4. DISCUSSION

Considering zones of inhibition as a measure of plant extracts activity, both the ether and ethanol extracts of *T. riparia* were more active against *C. albicans* than Nystatin. *T. riparia* has a great potential for the development of an antifungal agent. Previous ethnobotanical studies have mentioned some of these plant species to be used in treating fungal, bacterial or viral infections and other diseases in different parts of Africa [25-28]. The active compounds of *E. arabicum*, *S. manii* and *P. africanum* could also be a potential source of antifungal drugs if they are purified.

The low MIC values of *T. riparia* of 0.0156 and 0.0312 mg/ml, for the ether and ethanol extracts respectively are indicative of the high activity the plant given low concentrations are required to inhibit the growth of *C. albicans*. Whereas the ethanolic extract of *S. manii* showed no observable effects on the growth of *C. albicans,* its ether was active against. Similarly, the ether extract of *P. lactiflorus* showed no observable effects on the growth of *C. albicans* while its corresponding ethanolic extract was active.

Table 1. Diameters (mm) of zones of growth inhibition of the plant extracts on *C albicans*

Plant name	*T. riparia*		*E. arabicum*		*P. lactiflorus*		*S. manii*		*P. africunum*	
Plant extracts	DE	Et	DE	Et	DE	Et	DE	Et	DE	Et
Diameter of zone of inhibition (mm)	28	31	21	12	n.a	12	13	R	14	R

Key: DE – Diethyl Ether, Et – Ethanol; n.a – Not active, standard drug Nystatin 25 mm

Table 2. Minimum inhibitory concentration (MIC) of extracts (g/ml) on *Candida albicans*

Plant name	*T. riparia*		*E. arabicum*		*P. lactiflorus*		*S. manii*		*P. africunum*	
Plant extracts	DE	Et	DE	Et	DE	Et	DE	Et	DE	Et
MIC mg/g	0.0156	0.0312	0.0625	0.5	n.a	0.5	0.125	n.a	0.5	0.5

Key: DE – Diethyl Ether; Et – Ethanol, n.a – Not active

Table 3. Major Phytochemical compounds in the respective plant extracts

Plant name parts used	*T. riparia* leaf			*E. arabicum* leaf			*P. lactiflorus* leaf			*S. manii* stem & leaf			*P. africunum* root		
Plant extracts	**DE**	**Et**	**HE**	**DE**	**Et**	**HE**	**DE**	**Et**	**HE**	**DE**	**Et**	**HE**	**DE**	**Et**	**HE**
Phytochemicals															
Basic alkaloids	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-
Alkaloid salts	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyanin	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Coumarin derivatives	-	-	+	+	-	+	+	-	+	+	-	+	+	-	-
Emodols	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavone aglycones	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Flavanosides	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Higher fatty acids	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Reducing compounds	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
Steroid glycosides	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
1. Sterols & triterpenes	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
2. Tannins	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-

Key: Compound present (+), Compound absent (-), DE – Diethyl Ether, Et – Ethanol, EH – Hydrolysed ethanol

In general, ethanolic extracts of *T. riparia* and *P. lactiflorus* were found more active against *C. albicans* than their corresponding ether extracts. The ether extracts of *E. arabicum*, *P. afticanum* and *S. mannii* were more active than their corresponding ethanolic extracts. This implies that the active ingredients of *T. riparia* and *P. lactiflorus* are polar and therefore more soluble in a polar solvent while for *E. arabicum*, *P. africanum* and *S. mannii* their active ingredients are non polar and therefore more soluble in ether which is a non polar solvent. Different researchers have reported the use of these plant species for various ethnomedicinal uses; *T. riparia* has been reported to be taken as a leaf decoction once a day for de-worming in Kabale district, western Uganda [25]. The leaves are used for treating tuberculosis in Uganda [26], conjunctivitis, meningitis, malaria, skin diseases, stomach ache, ulcers, bilhazia, worms, rheumatism, dyspepsia, fever, colds, cough, toothache, kwashirko, blocked fallopian tubes in the Rwenzori region, western Uganda [27], febrile convulsions in south western Uganda [28], bacterial and fungal infections as well as herpes zoster in Queen Elisabeth Biosphere Reserve, western Uganda [8], cough and chest infections in the Eastern Cape province of South Africa (QUBS) [29].

The leaves of *Erucastrum arabicum* have been reported to be used for treating constipation and ringworm in western Uganda [30], red eyes in south western Uganda [27], while the roots used to treat diarrhea in Kenya [31]. A root infusion of *Plectranthus lactiflorus* is used to treat rheumatism [30] and bacterial and fungal infections in (QEBR), western Uganda [8]. A stem infusion of *Solanecio mannii* is drunk or bathed for relaxation of pelvic region for child birth in south western Uganda [27], whereas a root decoction *Platostoma africanum* is used as an aphrodisiac and for headaches and used in Tanzania [32].

4.1 Major Chemical Groups

Some phytochemical compounds already known to have antifungal properties were present in some of the plants screened. Tannins have been demonstrated to have a powerful antifungal action in clinical studies and also have wound healing and antiseptic effects [33,34], therefore, the tannins present in all the plant species analysed in this study could be responsible for their antifungal activity and can justify the ethnobotanical uses of the plant species by the local people. Cowan [35] also reported that several plant products utilized as antimicrobial agents to include quinones, flavonoids, tannins, coumarins, alkaloids and terpenoids. Flavonoids have also been shown to have antiviral, antiviral and antibacterial [36,37].

A GC/MS analysis of the essential oils from the leaves and stems of *T. riparia* from South Africa identified 35 components, with the main constituents being α-terpineol (22.6%), fenchone (13.6%), β-fenchyl alcohol (10.7%), β-caryophyllene (7.9%), and perillyl alcohol (6.0%) [38]. The yields of essential oils have also been have been shown to range from 0.17% to 0.26% [39].

5. CONCLUSION

The people of Bwindi use several medicinal plants to treat fungal infections. Generally, all plant species examined were active against *Candida albicans. T. riparia* exhibited the highest antifungal activity of all the plant species analysed with inhibition diameters zones of 28 and 31 mm for the ether and ethanol extracts respectively. Both these zones were higher than those exhibited by the positive control – Nystatin, which had a diameter of zone of inhibition of 25 mm. *T ripararia* also had the lowest MIC values for both extracts further confirming it potency even at low concentrations and therefore can guide in the development of alternative treatment for antifungal treatments especially in *Candida albicans*.

CONSENT

Prior informed consent as obtained from all the participants verbally before they were interviewed.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fleming RV, Walsh TJ, Anaissie EJ. Emerging and less common fungal pathogens. Infectious Disease Clinics of North America. 2002;16:915–933.

2. Garbino J, Kolarova L, Lew D, Hirschel B, Rohner P. Fungemia in HIV-infected patients: A 12-year study in a tertiary care hospital. AIDS Patient Care STDs. 2001; 15:407–410.
3. Hamza OJM, Van den Bout-van den Beuke CJP, Matee MI, Moshi JM, Mikx FHM, Selemani HO, Mbwambo ZH, et al. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. J Ethnopharmacol. 2006;108:124–132.
4. Liu Q, Luyten W, Pellens K, Wang Y, Wang W, Thevissen K, et al. Antifungal activity in plants from Chinese traditional and folk medicine. J Ethnopharmacol. 2012;143:772–778.
5. Mulaudzi RB, Ndhlala AR, Kulkarni MG, Finnie JF, Van Staden J. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. Journal of Ethnopharmacology. 2011;135: 330–337.
6. Freixa B, Vila R, Vargas L, Lozano N, Adzet T, Canigueral S. Screening for antifungal activity on nineteen Latin American plants. Phytotherapy Research. 1998;12:427–431.
7. Vazquez JA. Therapeutic options for the management of oropharyngeal and esophageal candidiasis in HIV/AIDS patients. HIV Clinical Trials. 2000;1:47–59.
8. Kamatenesi MM, Oryem-Origa H, Olwa-Odyek, Makawiti DW. Medicinal plants used in the treatment of fungal & bacterial infections in & around Queen Elizabeth Bioshpere Reserve, Western Uganda. African J Ecol. 2008;46(1):90-97.
9. Ponnusamy K, Petchiammal C, Mohankumar R, Hopper W. In vitro antifungal activity of indirubin isolated from a South Indian ethnomedicinal plant *Wrightia tinctoria* R. Br. J Ethnopharmacol. 2010;132:349–354.
10. Debruyne D. Clinical pharmacokinetics of fluconazole in superficial and systemic mycoses. Clinical Pharmacokinetics. 1997; 33:52–77.
11. Maregesi SM, Pieters L, Ngassapa OD, Apers S, Vingerhoets R, Cosc P, et al. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. J Ethnopharmacol. 2008;119:58–66.
12. Runyoro DKB, Ngassapa OD, Matee MI, Joseph CC, Moshi MJ. Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. J Ethnopharmacol. 2006;106:158–165.
13. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, Salem K, Al-Nuri MA: Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J Ethnopharmacol. 1998;60:265–271.
14. Njoroge GN, Bussmann RW. Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. Journal of Ethnopharmacology. 2007;111:303–307.
15. Svetaz L, Zuljana F, Derita M, Petenatti E, Tamayo G, Cáceres A, et al. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. J Ethnopharmacol. 2010;127: 137–158.
16. Olupot W, Baringiya R, Chapman: CA The status of anthropogenic threat at the people-park interface of Bwindi Impenetrable Forest National Park, Uganda. Environmental Conservation. 2009;36(1):41-50.
17. Forestry Department Uganda. Uganda National Biodiversity Report; 2000.
18. Martin GJ. Ethnobotany: A methods manual. Chapman & Hall, London; 1995.
19. Hue NV, Uchida R, Ho MC. Sampling and analysis of soils and plant tissues how to take representative samples, how the samples are tested from. In: Silva JA, Uchida R, Ho MC, editors. Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture. College of Tropical Agriculture and Human Resources, University of Manoa; 2000.
20. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books, Ibadan; 1931.
21. Trease GE, Evans WC. Trease and Evans: Pharmacognosy. 13th ed. Bailliere Tindale, London; 1989.
22. Ostrosky-Zeichner L, Bazemore S, Paetznick V, Rodriguez JR, Chen E, Wallace T, et al. Differential antifungal activity of isomeric forms of Nystatin. Antimicrobial agents and Therapy. 2001; 45(10):2781–2786.
23. Kakudidi E, Anywar G, Ayorekire F, Ogwal-Okeng J. Antifungal medicinal plants used by communities adjacent to Bwindi Impenetratable National Park, Uganda. European J Medicinal Plants. 2015;7(4): 184-192.

24. Hamill FA, Apio S, Mubiru NK, Mosango M, Bukenya-Zirab R, Maganyi OW, et al. Traditional herbal drugs of southern Uganda. Journal of Ethnopharmacology. 2000;70:281–300.
25. Bunalema L, Obakiron S, Tabuti JRS, Waako P. Knowledge on plants used traditionally in the treatment of tuberculosis in Uganda. J Ethnopharmacol. 2014;151: 999–1004.
26. Monik A. Family plant medicinal Garden in the Rwenzori region: Marianum Press Ltd Entebbe; 2005.
27. Ssegawa P, Kasenene JH. Plants for malaria treatment in Southern Uganda: Traditional use, preference and ecological viability. J Ethnopharmacol. 2007;27:110-131.
28. Ndamane Y, Kambizi L, Songca SP, Oluwafemi OS. Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system. J. Medicinal Plants Research. 2013;7(37):2755-2760.
29. Kakudidi EK, Bukenya-Ziraba R, Kasenene JM. The Medicinal Plants in and around Kibale National Park in western Uganda. Lydia Nowergian Journal of Botany. 2000;5:109-124.
30. Arikan S, Ostrosky-Zeichner L, Lozano-Chiu M, Paetznick V, Gordon D, Wallace T, et al. *In vitro* activity of Nystatin compared with those of liposomal Nystatin, Amphotericin B, and Fluconazole and around Kibale National Park in western Uganda. Lydia Norwegian Journal of Botany. 2000;5:109-124.
31. Njoroge GN, Kibunga JW. Herbal medicine acceptance, sources and utilization for diarrhoeal management in a cosmopolitan urban area (Thika, Kenya). African J Ecol. 2007;45(1):65-67.
32. Kowaro JO. Medicinal plants of East Africa 2nd. Ed Kenya Literature Bureau Nairobi; 1976.
33. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564-582.
34. Latte KP, Kolodziej H. Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. Zeitschrift fur Naturforschung. 2000;55:467–472.
35. Ndhlala AR, Muchuweti M, Mupure C, Chitindingu K, Murenje T, Kasiyamhuru A, Benhura, MA. Phenolic content and profiles of selected wild fruits of Zimbabwe: *Ximenia caffra, Artobotrys brachypetalus* and *Syzygium cordatum.* International J Food of Science and Technology. 2008;43: 1333–1337.
36. Pengelly A. The constituents of medicinal plants 2nd ed. An introduction to the chemistry and therapeutics of medicinal plants; CABI publishing Cambridge; 2004.
37. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A review. Tropical J of Pharmaceutical Research. 2008;7(3):1089-1099.
38. Campbell WE, Gammon DW, Smith P, Abrahams M, Purves T. Composition and antimalarial activity *in vitro* of the essential oil of *Tetradenia riparia.* Planta Med. 1997; 63:270–272.
39. Gazim CZ, Amorim ACL, Hovell ANC, Rezende CM, Nascimento IA, Ferreira GA, et al. Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of *Tetradenia riparia* (Hochst.) Codd in Southern Brazil. Molecules. 2010;15:5509-5524.

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