Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 03, pp.13099-13103, March, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

QUALITATIVE PHYTOCHEMICAL AND GC-MS ANALYSIS OF *TROCHOMERIA DALZIELII* SEED OIL

[1]Mankilik, M., [2]Mhya, D., [1]Mikailu, A. S., [3]Ringim, S.I., [1,*]Mohammed, S .Y.

[1.]Nigerian Institute of Leather and Science Technology (NILEST) Zaria, Nigeria
[2.]Department of Biochemistry, Ahmadu Bello University (ABU) Zaria, Nigeria
[3]Department of Biochemistry, Federal University Dutse (FUD), Nigeria

ARTICLE INFO

Article History:
Received 21st December, 2014
Received in revised form
30th January, 2015
Accepted 25th February, 2015
Published online 17th March, 2015

Key words:

Trochomeria dalzielii, Seed oil, Phytochemicals, GC–MS

ABSTRACT

Qualitative phytochemical analysis of *Trochomeria dalzielii* seed oil was carried out with the aim to provide analytical data of the seed oil for its medicinal and/or nutritional importance. The *T. dalzielii* seed oil was extracted using Soxhlet extractor. Fatty acids composition of *T. dalzielii* seed oil was determined by GC–MS technique while the phytochemical analysis was done by standard methods. The fatty acids compositions revealed six bioactive components including; Oleic, Linoleic, Palmitic, Stearic, Eicosanoic, and Behemic acids. Oleic acid (57.95%) is the most abundant and Behemic acid (0.47%) is the less. The result of the phytochemical analysis of *T. dalzielii* seed oil revealed the presence of flavonoids, saponins, tannins, alkaloids, steroids, resins and glycosides. Several studies have reported the importance of these phytochemicals, and based on these findings, it could be suggested that *T. dalzielii* seed oil might serve as potential nutraceuticals.

INTRODUCTION

Medicinal plants are plants that are useful in folk medicine as a remedy for many diseases and ailments such as fever, rheumatism, gout, diarrhea, dysentery, gastrointestinal pain and disorder. They constitute the major constituents of most indigenous medicines and a large number of western medical preparations contain one or more component (s) of plant origin (Riaz *et al.,* 2012). A number of plants have been investigated for their biological activities. Recently, interest has increased considerably in finding naturally occurring plant products for use in foods or medicine to replace synthetic chemicals. In addition, natural antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to the consumers (Riaz *et al.,* 2012). The plant *Trochomeria dalzielii* belongs to the *cucurbiteaceae* family is a herbaceous annual liane of about 2.5m long. It is a deciduous plant of the woodland, bush land and savannah of eastern Senegal, northern Nigeria, Sudan, Ethiopia, Tangayinka and southwest Africa (Burkill, 1985). The seed of *T. dalzielii* is ovoid, smooth, round, and humid enclosed in pockets of whitish jelly testa. The literature survey on the family shows that the plants have been used for various health benefits.

Corresponding author: Mohammed, S .Y.
Nigerian Institute of Leather and Science Technology (NILEST) Zaria, Nigeria

Cucurbitaceae family is composed of pumpkin, gourds and melons (Lima *et al*., 2010). Many of the family members have been reported to be cultivated for their medicinal and nutritional properties (Milinda and Kaur, 2011). *T. dalzielii* have been used in agri-hortculture, food, medicine, veterinary medicine and other products. Medicinal plants contain physiologically active constituents, which over the years have been exploited in traditional medical practice for the treatment of various ailments (Mohammed *et al*., 2013). The medicinal value of plants lies in some chemical substances called phytochemical that produce a significant physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Amin *et al*., 2013). Phytochemicals are bioactive non nutrient chemical compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They are secondary metabolites that contribute to flavor and color. Many phytochemicals have antioxidant activity and reduce the risk of many diseases. Their functions and mechanisms of action may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antimicrobial properties (Mohammed, 2013). Many diseases are associated with oxidative stress caused by free radicals. Free radical species are pivotal for the onset of various disease conditions such as hypertension, atherosclerosis, cancer and alzheimers disease. However, phytochemicals protect cells against the damaging effects of free radical species such as singlet

oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxy nitrite through their antioxidant property (Manisha *et al*., 2012). In the same vein, the use of secondary metabolites have also shown to be increasing at a higher rate by the industries especially in food production, amino acids, development of vaccines and antibiotics, among others (Kristen, 2012). Seed oils represent some of the earliest cosmetic products used by humans. For decades, many of these oils used for cooking and eating have also been used for healthy skin and hair. However, modern research has uncovered that these nutritional seed oils don't just contain lipids but bioactive lipids called essential fatty acids. Essential fatty acids (EFAs) are fatty acids that are required for normal health and wellbeing of the body but must be obtained through the diet, as we cannot synthesize them. There are two families of EFAs: omega – 3 and omega – 6 (Aslam *et al*., 2006). The nutritional and health benefits of fatty acids are extensive. They are essential in the manufacturing and repair of cell membrane, enabling cells to obtain optimum nutrition, expel harmful waste products. A primary function of essential fatty acid is the production of prostaglandins, which regulate body functions such as heart rate, blood pressure, fertility and play a role in immune function by regulating inflammation and encouraging the body to fight infections (Simopoulos, 1991). Phytochemicals offers great hope for the control of chronic human diseases. The role of phytochemicals in human health and diseases is still a fertile area of research. Therefore, this paper investigate the phytochemicals and fatty acid constituents of *T. dalzielii* seed oil with the aim to provide analytical data of the seed oil for medicinal and/or nutritional importance.

MATERIALS AND METHODS

Sample collection

The seeds of *Trochomeria dalzielii* were procured from Samaru local market in Zaria. Zaria is located in Kaduna State, northern part of Nigeria. The seeds were grounded into fine powder using laboratory pestle and mortal, and then use for analysis.

Extraction

About 40g of the powdered *Trochomeria dalzielii* seed were placed into the Soxhlet thimble and extracted with methanol (96%). The solvent was concentrated in the vacuum and allowed to dry in the fume hood to afford the oil. The oil was kept at 4^{o}C, and then later used for phytochemical and fatty acids analysis.

Phytochemical Analysis

The phytochemical analysis of *T. dalzielii* methanolic extracts was carried out by standard methods as described by (Sofowora, 2008; Evans, 2002; Harbone, 1973).

Test for Alkaloids

A quantity (0.2g) of the sample was boiled with 5ml of 2% HCl on a steam bath. The mixture was filtered and 1ml portion of filterate was measured in to 2 test tubes each 1ml was treated with mayers reagent. A creamy white colored precipitate indicates the presence of alkaloids. Wagners reagent: a reddish brown precipitate indicates the presence of alkaloids.

Test for Flavonoids

A quantity (0.2g) of extract was mixed with 1ml of 10% NaOH. A resulting yellow coloration indicates the presence of flavonoids.

Test for Glycosides

Dilute sulphuric acid was added to 0.1g of the test extract in a test tube and boiled for 15 min in a water bath. It was cooled and neutralized with 20% potassium hydroxide solution. A mixture 10ml of equal parts of Fehling's solution A and B are added and boiled for 5minutes. A more dense red precipitate indicates the presence of glycoside.

Test for Saponins

A quantity (0.1g) of the extracts was boiled with 5ml of distilled water for 5 min. The mixture was filtered and allowed to cool. A quantity (1ml) of the filterate was diluted with 4ml of distilled water. The mixture was shaken vigorously and observed on standing for a stable froth.

Test for Resins

The precipitate test was carried out to detect the presence of resins in the plant. A quantity (0.2g) of the extract treated with 15ml of 96% ethanol. The alcohol extract was then poured in to 20ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Test for Steroids

A quantity 2ml of chloroform was added to 0.1g of extract to which few drops concentrated sulphuric acid was added, a reddish brown precipitate indicates the presence of steroids.

Test for Tannins

A quantity (1g) of the sample was boiled with 20ml distilled water for 5 min in a water bath and was filtered while hot. One (1ml) of cool filterate was added to 5ml distilled water and a few drops of 10% ferric chloride were added. A bluish – black or brownish – green precipitate indicated the presence of tannins.

GC – MS Analysis

The fatty acids composition of *T. dalzielii* seed oil was analyzed by gas chromatography instrument (QP2010 plus) equipped with FID detector and high temperature HT5 AQ (SQE) 12m x 0.22mm column. The column flow was 1.80 ml/min, purge flow was 3.0 ml/min and total flow was 40.8 ml/min. The injector temperature was 250^{o}C/min; oven temperature was programmed from 70^{o}C to 280^{o}C at 10^{o}C/min and was held at 250^{o}C for 5min. The oil was converted to fatty

acid methyl esters (FAME) using potassium hydroxide/methanol method (Liu, 1994). The FAME was extracted and dissolves in hexane before subjecting for the GC–MS analysis.

RESULTS AND DISCUSSION

The phytochemical analysis of *T. dalzielii* seed oil reveals the presence of alkaloids, flavonoids, glycosides, resins, saponins, steroids, and tannins (Table 1).The results indicated high concentration of saponins, moderate concentration of flavonoids, steroids and tannins, while the concentration of alkaloids, glycosides and resins are low. It is interesting to note that, phytochemical analysis on the *curcurbitaceae* family have shown the presence of similar secondary metabolites (Gills *et al.,* 2012).

Table 1. Phytochemical screening of methanolic extract of *T. dalzielii* seed oil

Phytochemicals	Inference
Alkaloids	+
Flavonoids	++
Glycosides	+
Resins	+
Saponins	+++
Steroids	++
Tannins	++

Key: - = Not detected, + = present in low concentration,
++ = present in moderate concentration,
+++ = present in high concentration.

Table 2. Fatty acids profile of methanolic extract of *T. dalzielii* seed oil

Fatty acids	Percentage (%) composition
Oleic	51.62
Linoleic	12.87
Palmitic	16.95
Stearic	09.64
Eicosanoic	01.95
Behemic	00.47
Others	06.50

The natural plant products that have received greatest attention with regards to possible medicinal applications are alkaloids and saponins (Mohammed *et al*., 2013), however our result revealed the presence of saponins in high concentration which therefore justify the medicinal properties of this plant. Several studies have reported the importance of phytochemicals; in particular, flavonoids, saponins and tannins were reported to be responsible for the antimicrobial properties of some ethnomedicinal plant extracts (Singh and Bhat, 2003). Alkaloids have analgesic, anti – inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994; Sanni *et al.,* 2008). Alkaloids were also reported to have antimicrobial properties against both Gram – positive and Gram – negative bacteria (Sunilson *et al.,* 2009). Flavonoids play a significant protective role against oxidative stress-mediated human diseases as well as antiulcer and anti-inflammatory activities (Gills *et al*., 2012). In addition, flavonoids have been referred to as nature's biological response modifiers following strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens (Evans, 2002). Glycosides have been found to play significant important role in the treatment of congestive heart failure as well as cardiac arrhythmia. Cardiac glycosides works by inhibiting Na^+/K^+ pump thereby increasing Sodium ions level in the monocytes, which in turn raises the level of Ca^{2+}. Increase in Ca^{2+} favors the contraction of the heart muscle by improving cardiac output and also reduces distention of the heart (Ngbede *et al*., 2008). Saponins are also known for their medicinal properties as a natural blood cleanser, expectorant and antibiotics. Their expectorant action is very useful in managing upper respiratory tract inflammation. Saponins present in plants are cardiotonic in nature and also have antidiabetic properties (Sanni *et al*., 2008). Tannins are known for their astringent property which hasten wound healing and ameliorate inflamed mucus membrane, antimicrobial activity, anti-inflammatory, and anti-diarrheal properties (Sanni *et al*., 2008; Palomb, 2006). Also plant tannins have been used as a source of commercial tannic acids and tannin agents (Evans, 2002).

The fatty acids composition of *T. dalzielii* seed oil determined by the GC–MS analysis have revealed six bioactive components including; Oleic acid, Linoleic acid, Palmitic acid, Stearic acid, Eicosanoic acid and Behemic acid (Table 2). The major constituent of the oil were Oleic acid (51.62%), Palmitic acid (16.95%), Linoleic acid (12.87%) and Stearic acid (9.64%) while Eicosanoic acid (1.95%) and Behemic acid (0.47%) are the minor constituents. Figure 1 is the chromatogram of *T. dalzielii* seed oil showing 13 notable peaks which represents the amino acid constituents of the analyte. The percentage concentration of the amino acids was obtained by spectrum comparison of the peak report generated by the GCMS instrument and a library also obtained from the instrument. From the library peaks 1 and 2 of Figure 1 represents Palmitic acid, and from the peak report the % area of peaks 1 and 2 are 7.14 and 9.81, adding them up gives 16.95. This suggests that the % concentration of Palmitic acid in the analyte is 16.95%. Peak 3 represents Linoleic acid and the % area of the peak (from peak report) is 12.87, hence the concentration of Linoleic acid is 12.87%. Peaks 4 and 6 represents Oleic acid (from the library) and % area (from peak report) are 12.10 and 39.52 which translate to concentration of 51.62%. Peaks 5 and 7 represents Stearic acid (from the library) and % area are 2.94 and 6.70 (from peak report), adding them gives 9.64% as the concentration of Stearic acid.

Peaks 8 and 9 represents Eicosanoic acid (from the library) and % area are 1.34 and 0.61 (from peak report), adding them up gives 1.95% as the concentration of Eicosanoic acid. Peak 12 represent Behemic acid (from the library) the % area is 0.47% which is the concentration of Behemic acid in the oil. Peaks 10, 11 and 13 represent other fatty acids. Therefore, this suggests that the oil could be a better source of Oleic acid. It is noteworthy to know that Oleic, Palmitic, Stearic and Linoleic acids have been found in abundant in plant seed oil such as *Palp* seed oil (Shadi *et al*., 2013), *Hevea brasiliensis* seed oil and *sumbucus* species seed oil. The presence and composition of the fatty acids in *T. dalzielii* seed oil as determined in our study is consistent with the above findings. Omega – 3 and Omega – 6 fatty acids have been reported to have beneficial qualities.

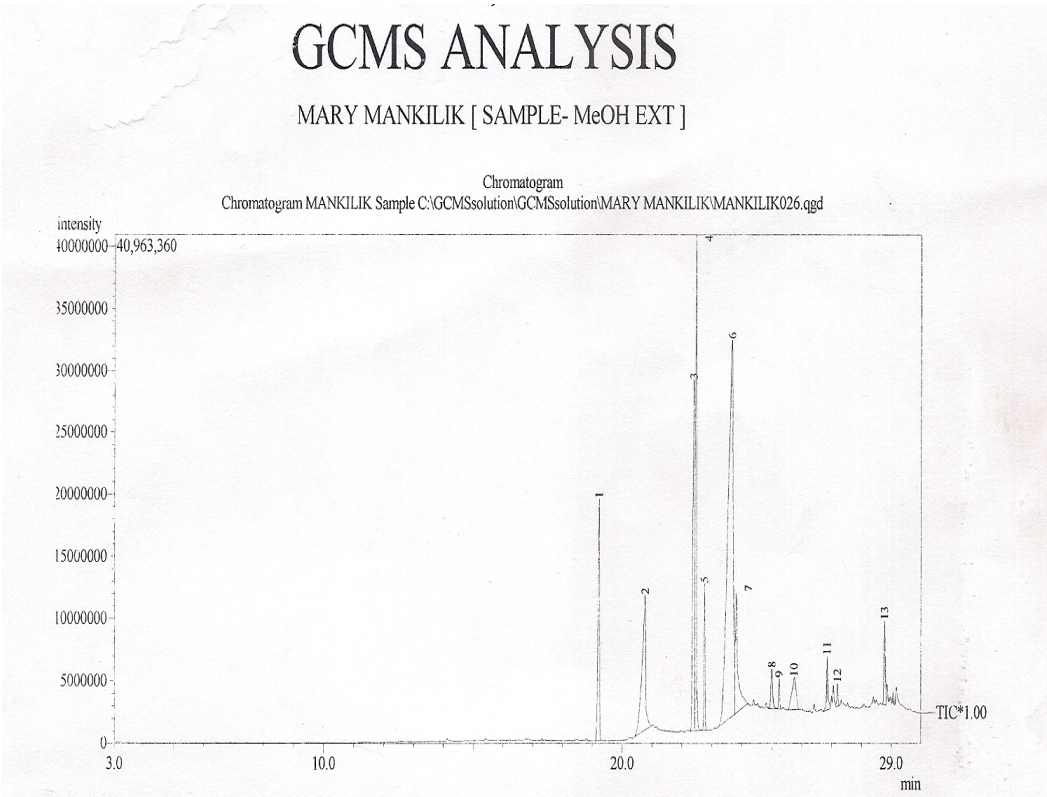

Figure 1. Chromatogram of *T. dalzielii* seed oil showing fatty acids composition

Source: GCMS Instrument – GCMS-QP2010 PLUS – SHIMADZU JAPAN

Omega – 3 fatty acids have an inhibitory effect on cancer and tumor growth.n addition to their anticancer properties omega – 3 fatty acids have been shown to lower blood pressure and blood cholesterol levels, help normalize fat metabolism and decrease insulin dependence in diabetics, increase overall metabolic rate and membrane fluidity and exhibit anti – inflammatory properties, specifically with regard to relieving arthritis (Leizer *et al.*, 2000). While there are many sources of essential fatty acids in the diet, *T. dalzielii* seed oil has proven to be exceptionally rich in some of these compounds.

Conclusion

The study is able to report that *T. dalzielii* seed oil contain compounds such as flavonoids, saponins, tannins, glycosides among others as well as significant quantity of some essential fatty acids such as oleic acid. Based on these findings, it could be suggested that *T. dalzielii* seed oil might serve as potential nutraceuticals.

REFERENCES

Amin, M.M. Sawhney, S.S. and Jassal, M.M.S. 2013. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale.* Wudpecker *J. Pharm. Pharmacol*. 2(1): 1 – 5.

Aslam, M.N. Lansky, E.P. and Varani, J. 2006. Pomegranate as a cosmeceutical source: pomegranate fractions promote proliferation and procollagen synthesis and inhibit matrix

Burkill, H.M. 1985. The useful plant of west Tropical Africa. 2nd Edition Royal Botanic Gardenkew, pp 146 – 49.

Evans, W.C. 2002. Trease and Evans Pharmacognosy. 15th Edition. Elsevier, India, pp 27 – 486.

Gill, N.S. Pooja, R. Arora, R. Dhawan, V. and Bali, M. 2012. Evaluation of antioxidant, anti-inflammatory and antiulcer potential of *Mormodica charantia* methanolic seed extract. Res. *J. Phytochem.,* 6: 96 – 104.

Gupta, S.S. 1994. Prospects and perspectives of natural plant products in medicine. *Indian J. Pharmacol*., 26: 1 – 12.

Harbone, J.B. 1973. Phytochemical screening methods: A guide to modern techniques in plant analysis. Chapman and Hall, London., pp 411 – 15.

Kristen, W. 2012. Classification of secondary metabolites, How plants and humans use them. Available at: http://www.interactivebiology.com

Leizer, C. Ribnicky, D. Poulev, A. Dushenkov, S. and Raskin, I. 2000. The composition of Hemp seed oil and its potential as an important source of nutrition. *J. Nutr. Funct. Med*. Foods., 2(4): 35 – 53.

Lima, L.F.P. Evaldt, A.C.P. Bauermann, S.G. and Miotto, S.T.S. 2010. Pollen morphology of Brazilian *Fevillea cucurbitaceae*. Grana., 49: 263 – 68.

Liu, K. 1994. Preparation of fatty acid methyl esters for Gas – chromatographic analysis of lipids in biological materials. *J. Am. Oil. Chem*. Soc., 71(11): 1179 – 87.

Manisha, B. Anjoo, K. and Ajay, K.S. 2012. Phytochemical screening and in – vitro evaluation of antioxidant activities of various extracts of leaves and stems of *Kalenchoe crenata. J. Pharm*. Res., 5(8): 3967 – 72.

metalloproteinase – 1 production in human skin cells. *J. Ethnopharmacol*. 103: 311 – 18.

Milinda, P. and Kaur, S. 2011. Is bottle gourd a natural gourd? *Int. Res. J. Pharm*., 6: 13 – 17.

Mohammed, A. Liman, M.L. and Atiku, M.K. 2013. Chemical composition of the methanolic leaf and bark extracts of *Senna siamea* Lam. *J. Pharmacognosy Phytother*., 5(5): 98 – 100.

Mohammed, S.Y. 2013. Quantitative phytochemical and elemental analysis of *Guiera senegalensis* leaf extract. J. Pharmacognosy Phytother., 5(12): 204 – 207.

Ngbede, J. Yakubu, R.A. and Nyam, D.A. 2008. Phytochemical screening for active compounds in *Cananum schein furthic* (Atile) leaves from Jos North, Plateau state, Nigeria. Med. *Res. J. Biol. Sci*., 3(9): 1076 - 78.

Palomb, E.A. 2006. Phytochemicals for traditional medicinal plants used in the treatment of diarrhea: Modes of action and effects on intestinal function. *Phyto. Rev*., 20: 717-24.

Riaz, T. Abbasi, A.M. Rehman, A. Shahzadi, T. Ajaib, M. Khan, M.K. 2012. Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscose* Jacq. *J. Serb. Chem. Soc*., 77(4): 423 – 35.

Sanni, S. Onyeyili, P.A. and Sanni, F.S. 2008. Phytochemical analysis, elemental determination and some in vitro Antibacterial Activity of *Ocimum basilicum L*. leaf extracts. *Res. J. Phytochem*., 2(2): 77 – 83.

Shadi, S. Hamed, M. Chin, P. T. and Hassanah, M.G. 2013. Ultrasound–assisted extraction and solvent extraction of papaya seed oil yield, fatty acid composition, triacylglycerol profile. *Molecules.,* 18(10): 12474 – 87.

Simopoulos, A.P. 1991. Omega – 3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.,* 54: 438 – 63.

Singh, B. and Bhat, T.K. 2003. Potential therapeutic application of some anti-nutritional plant secondary metabolites. *J. Agric. Food Chem*., 51: 5579 - 97.

Sofowora, A. 2008. Medicinal plants and Traditional plants in West Africa. 3rd Edition. Spectrum Books Ltd. Ibadan, Nigeria., pp 199 – 204.

Sunilson, J.A. Suraj, R. Anandarajagopal, K. Rejitha, G. Vignesh, M. and Promwichit, P. 2009. Preliminary phytochemical Analysis, elemental determination and antibacterial screening of *Codium decorticatum* Marine Green Algae. *Int. J. Biol. Chem*., 3(2): 84 – 89.

****