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Essential oil components of *Hydrocotyle verticillata* leaf

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Abstract
Essential oils (EOs) and phytochemicals have been noted to possess useful source of essential chemical substances which are made use of in numerous fields of study including in cosmetics industry, medical research, pharmaceutical industry, and food and beverage industry as well as in agric subsector. These phytonutrients have been discovered as a notable alternative for the treatment and prevention of a wide myriad of diseases plaguing man for some centuries. The extraction and characterization of Eos is the most important step in the identification of these plant chemical compounds. This study investigated the essential oil components in *Hydrocotyle verticillata* leaf. The dried leaf extracts of the plant was procured based on standard protocol and the essential oil extracted using Hydro-distillation method while the various components were identified and quantified using GC-MS from Agilient Technology. The identified components includes estragole [66.85%], 3. beta.17. beta.-dihydroxyetr-4-ene [10.89%], 1,3,7-octatriene, 3,7-dimethyl [9.70%], Hexadecanoic acid, ethyl ester [7.49%], and 1-methylbicyclo(3.2.1) octane [7.28%] in that order and have shown to possess anti-inflammatory, anti-oxidant, anti-cancer and neuro-stimulatory properties among others.

Keywords: Essential oils, *Hydrocotyle verticillata*, GC-MS analysis, Hydro-distillation, anti-cancer

1. Introduction
The awareness about plants products is on the increase which could be attributed to the ever increasing demand on them for treatment of tropical diseases due to their low side effects. Aside the rich pharmacological components in these essential oils, Eos finds applications in other fields such as cosmetics and perfumery industry [1]. It is pertinent to outline a formidable strategy and to have a good view of the phytochemistry and biological importance of these phytonutrients together with their role in human wellbeing and nutritional potentials. Odo *et al.*, [8] noted the beneficial role of essential oils and asserted that they are composed of complex and multifunctional metabolites inherent in plants and have found usefulness for the past years in prevention and treatment of numerous metabolic derangements. Odo *et al.*, [8] posited that EOs are aromatic plants secondary substances serving several function which includes protection from herbivores, to chemicals plants used in communicating among same species and intracellular signaling within plants as a result of environmental needs [1]. It has been noted that individual plant has evolved strategy to protecting and preserving itself from invaders with the use of specific components of essential oil chemical constituents [1]. It is worthy to note that these could contain from 20-60 constituents at varying concentration containing two or three chemical components of about 20-70% [8].
It has been estimated that about 3000 essential oils extracted from more than 2000 variety of plants comprising of not less than 300 of them possess known medicinal characteristics. Essential oils can be subdivided into hydrocarbon and oxygenated compounds based on their chemical similarities [1]. The compound that comprises ketones, phenols, oxides, esters and aldehydes are collectively belonged to the oxygenated essential oils unlike the other class which is composed of sulphur and aromatic containing compounds. Essential oils containing hydrocarbons are made up of known chemical compounds collectively known as terpenes. They comprises of different numbers of isoprene units [C-5]. The monoterpenes [C-10] as well as the sesquiterpenes [C-15] could be termed as being the classical terpenes. Even-though the class that contain isoprene units could also contain diterpenes [C-20]. The monoterpenes have been discovered to contain up to 90% of essential oils as a baseline constituent [3]. The components comprising monoterpenes and sesquiterpenes contain numerous structures having

known biomedical active functional centers as well as chemical orientation with more of oxygenated compounds [3]. The remaining components are aldehydes, ketones as well as alcohols which is composed of a huge number of aromatic metabolites involving aromatic compounds known to either be fruity ([E]-nerolidol), floral [Linalool], citrus [limonene], as well as herbal origin [γ-selinese] [1, 4]. Essential oils of plant origin equally comprise the group that contain non-terpenoid biogenated metabolites by a pathway termed phenylpropanoids which includes cinnamaldehyde, eugenol, as well as safrole [2]. It has been known that they possess variable composition in qualitative and quantitative fashion with various factors which are known to be responsible for the grouped intrinsic factors. It is noteworthy to state that this could be linked to the close proximity and interaction that exists between the plant and the environment where it is located, together with the maturity of the essential oil bearing plants concerned. It is important to state that even during harvest, the extrinsic factors and the day are very much related to the method of extraction employed as well as the environment.

Essential oil yield and the its composition rely on certain factors which are many even-though in certain instances, it could be tasking to segregate these factors from each other stemming from the fact that they are connected and are influenced by each other [2]. These factors include the plants parts, the degree of the plant maturity, seasonal changes, genetic variation as well as geographical locations.

There are varieties of methods designed to extract and isolated volatile compounds from aromatic plants [2]. The widely employed protocol is the steam distillation approach enunciated by Cocking and Middleton which were cited in the European Pharmacopoeia as well as in many other literatures [2, 4].

1.1 Roles of essential oils

It has been known that essential oils belonged to the class termed secondary metabolites with a pivotal important in guarding plants against intruders and these comprises majorly of terpenes with determined volatile speciation and having wide range of chemical orientations. Very worthy to note is the wide range of medical applications such as anti-microbial role that have been attributed to essential oils in treating many human impairment and disease states [3]. Essential oils have been speculated to attenuate cancer growth through multiple mechanism of action either through synergistic or antagonistic route. It has been noted that essential oils exerts its action by targeting cancer cell growth particularly signaling pathways [8]. The known regime include chemotherapic drugs such as paclitaxel and Doxetaxel which have been acclaimed to possess proimmune roles when utilized in cancer treatment [2]. Noteworthy is the fact that only about 5-15% plants from higher order have been characterized for their bioactive metabolites. Based on the fact that essential oils have been noted to be co-evolutionary substance originating from plants, its role to guard the species from invading herbivore makes them to release toxins which of course could be injurious to humans likewise other animals leading to altered body functions. It can be stated that these side effects of essential oils makes it possible to utilize them in disease treatments.

These include laxatives as well as emetics, cardiac stimulants, muscle relaxants, cardiac depressants which gives rise to reduced blood pressure and onset of bradycardia. Arterial hardening which results from fat accumulation in the arterial walls together with other compounds that aids in onset of thrombosis as well as arterial blockage could be relieved using essential oils [2].

It have been noted that the foremost cause of this ailment could be due to the oxidation of Low Density Lipoproteins [LDL-C] that carries bad cholesterol fractions, and it is evident from foregoing that phenolic containing essential oils which comprises eugenol and thymol shows the extreme LDL-C anti-oxidation effect by acting to reducing LDL-C affinity through the reduction of its affinity for the LDL-C receptor [4].

Additional treatment goal for cardiac abnormality could be alleviating the risk of arterial hardening which involves cholesterol reduction together with triacylglycerol plasma percentage wherein, it has been noted that black cumin oil caused a significant reduction in lipid levels in albino Wistar rats administered with the extract for about 12 weeks, giving a reduced adverse side effects to the renal as well as the hepatic function [4].

In addition, new research findings have shown that essential oils possesses anti-inflammatory and cytotoxic properties as much as exerting strong response to cardiovascular abnormalities through the prevention of the release of pro-inflammatory metabolites by decreasing Lipopolysaccharide levels [LPS] [8]. From the foregoing, it is evident that the ability of essential oils to combine with multiple cellular receptors gives its therapeutic role and thus, projecting the potential of EOs to be utilized in the treatment of debilitating diseases together with hidden internal diseases. Very much important is the role of EOs to activate multiple mechanisms giving rise to its response to metabolic and cellular stimuli and thus project it as a noble source of anti-carcinogenic therapy.

A form of alternative medicine termed aromatherapy has utilized the role of essential oil in its model where healing signs are given to compounds of aromatic nature. This phenomenon may be useful in causing the relaxation [8]. Essential oils when not utilized in proper manner could be detrimental to health. Among the various symptoms includes inflammation and skin irritation, allergic reaction [3]. A holistic review of about 201 indexed studies on essential oils acting as alternative therapy have shown that only about 10 have a well-documented and acceptable protocol and even most had some errors in references.

Work carried out on the role of essential oils as a potential natural pesticide have been promising and in other researches, some essential oils showed pest deterring effect particularly the arthropods and some insect vectors [4]. This could be observed in form of preventing digestion, repelling effect, reducing procreation rate, ability to achieve stunting growth, or demise of the invading insect vector that rely on the plant bearing essential oils. It is instructive to note that most substances exhibiting these features in the EOs enumerated above have been shown to be harmless for higher primates including humans [8]. This features projects their widespread use as green pesticides having no deleterious effect except on insect vectors. The mostly investigated plants bearing essential oils are lemon grass, Lavender, peppermint, eucalyptus, thyme and rose respectively.

Recent research on the role of EOs as a neoplastic treatment regime is relatively novel with most of the drugs used as chemotherapic agents coming from plant while about 25% others are mostly plants derived metabolites and another 25% as chemical prototype of phytonutrients [3]. Evading cell growth suppressor genes, bypassing of signaling pathways involved death and preventing either apoptotic cell death have

been the major milestone in cancer biology [4]. Any platform designed to stop programmed cell death together with attenuation of cellular progression is of immense importance. Studies have implicated EOs components as a key driver in battling neoplastic diseases by exhibiting different cellular roles such as acting on the cancer cell itself, together with various signaling pathways involved in the onset neoplastic diseases [4]. The anti-mutagenic and detoxification enhancement role of essential oils in addition to its anti-oxidant capability results in complete halt on the cancer signal leading to cellular proliferation even though other pathways in its formation are still vague. Essential oils role as anti-cancer agent equally includes the prevention and reduction of enzymes implicated in drug metabolic apoptotic pathways.

β- caryophyllene, eugenol acetate, carvacrol, Linalool, Thymol, geraniol acetate, bicyclogermcrene, cinnamaldehyde, geranial, neral have been noted to be the most anti-microbial application of essential oil components. Others include α-thujone, viridiflorol, limonene, (z) – linalool oxide, α- pinene, p-cymene, methyl chavicol, methyl cinnamate and (E) caryophyllene. Studies have been visible in show-casing essential oil role as a good anti-microbial agent with recent work on food incorporation *in vitro* evaluation recently undertaken [3-4].

1.2 *Hydrocotyle verticillata*

Hydrocotyle is a plant genus from the Apiaceae family and is made up of more than 100 other subspecies spread across both Tropical and Temperate areas of the World [5]. As reported by Odo *et al.*, [8], *Hydrocotyle verticillata* is frequently ascribed the name Whorled pennywort due to its flowering nature and is home to the America continents. It could also be found in western African state of Nigeria as reported by Odo *et al.*, [8], and West Indies inclusive.

The main identification features includes the possession of half dollar leaves that do show a compressed appearance of green bushes [6], with most of it showing a creeping features. Unlike other genus of the plant namely the *Hydrocotyle leucocephala*, *H. verticillata* creeping nature is shown mostly in aquarium and a perfect attraction when utilized as floor covering.

H. verticillata is known as a perennial, soft herb with length of about 10-15cm long and shows a creeping nature of the stem while its roots bear long stolon at the nodes.

Hydrocotyle verticillata possess a flexible leaf structure while its margins are coarse with its inflorescence being long of about 25 cm coming mostly from the axial side of the plant. The leaf of *H. verticillata* is mostly green in color and sometimes, it could be creamy white in appearance. Perfect growth is achieved when light is adequate with extreme pH range. *H. verticillata* plant does not rely to be rooted as it be equally utilized as a floating plant. The indoor application of the plant has equally been documented [6-7].

1.3 Geographical Distribution of *H. verticillata*

The plant *H. verticillata* has been reported by Odo *et al*., [8] to be mostly domiciled around the Americas but could be equally found in West African country of Nigeria where the author is located as well as in West Indies. It has been confirmed to be a perfect flowering plant found mostly in the warm areas. The plant seems to be found predominantly around the moist vegetation where it thrives well within shallow water [9]. In the Indian subcontinent, there appears to be other varieties of the genus Hydrocotyle which includes H. javanica Thumb, H. *burmanica* Kurz. H. *conferta* Wight, H. *rotundifolia* Roxb, and H. *asiatica* L. [8]. In a recent report, a sample of the genus Hydrocotyle which was sourced from Azimpur region of Dhaka city was identified as *Hydrocotyle verticillata* Thumb after painstaking effort with the active participation of Johnson & Smith, [9] which was brought forward by Khatun *et al.*, [7].

Plate 1: *Hydrocotyle verticillata* Plant [8]

1.4 Medical Importance of *Hydrocotyle verticillata*

Plant extract are frequently used for various ailment due to its low risk and greater efficiency. This has led to the development of anti-microbial agents from plants extracts having high propensity to target vital metabolic pathway inherent in bacteria [6-7]. Researchers are taking more interest in plant and its products as an easy and affordable source of medicine by isolating and characterizing these products for man's use. These active metabolites inherent in plants including essential oils promises to lead to greater path to discovering novel therapeutic compounds. These metabolites, showed more potential in recent years owing to the evolving nature of diseases and the onset of new ones in addition on how to prevent them [7]. Odo *et al*., [8], posited that *Hydrocotyle verticillata* were wrongly identified as Gotu kola which was the name given to it in the Niger Delta within Nigeria. The plants was reported to be used by the locals in the treatment of high blood pressure, inflammatory conditions, anemia of chronic origin, asthma, fever, urinary infection as well as enlarged spleen all of which were linked to *Centella asiatica* [6].

In traditional practice, the juice from *H. verticillata* is frequently utilized in the treatment of high temperature, as well as healing of boils and wound respectively. The treatment of bronchial inflammation, hepatic inflammation caused by viruses and renal problems has relied on the plant in the olden days. Efforts were equally being made to investigate its role as anti-carcinogenic, anti-oxidant, and arresting cellular proliferation [8].

2. Material and Methods

2.1 Collection and Naming of the Plant

Odo *et al*., [8] reported in their earlier article published in this journal how the plant was collected, identified and deposited in botany department of the University for Reference Purpose.

2.2 Formulation of the powdered extract of the plant

The entire leaf of the plant which was sourced by Odo *et al*., [8] as reported in a recent article on this Journal was subjected to a standard protocol on the use preparation of plant extract for analysis.

2.3 Protocol of essential oil extraction using Steam Distillation

100-150 g powdered extract of *H. verticillata* leaf was placed inside a 1-litre capacity distillation flask that was linked to a steam generator through a glass tubing and then to a condenser to collect the essential oil. The extraction conditions were placed at 100 °C for about 5 to 10 hour time limit. The mixture extracted was made to cool at room temperature while the oil was separated using a special separating funnel. The retrieved essential oils were then placed in a special designed container ready for characterization using combined gas chromatography and mass spectrometry apparatus.

2.4 Determination of essential oils using GC-MS analysis

GC-MS protocols were used for the characterization of the extracted essential oil of *H. verticillata* leaf from this study. Gas chromatograph from Agilient Technologies United State of America, have specification of 7890 [B] was combined to a mass spectrometer from same Agilient Technologies having the model of 5975[B] [8]. The protocol adopted was that enumerated by Odo *et al*., [8].

The review of the results obtained was carried out by utilizing information housed with an international agency as reported by Odo *et al*., [8] that comprises well above 6500 details [11]. The obtained information known as the spectrum from the analyzed essential oil in comparison with the standard was made use of in arriving at the final characterization of the essential oil components as presented in the result.

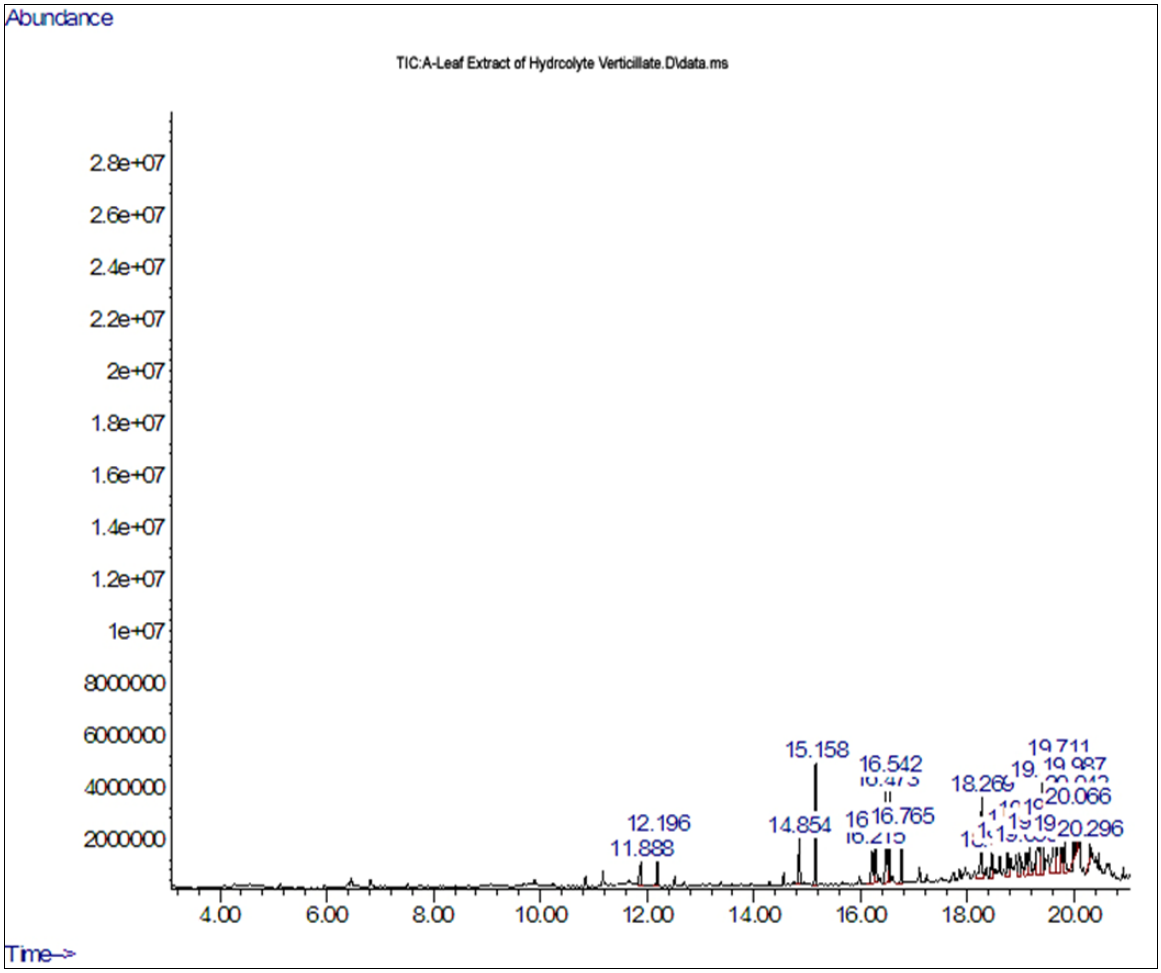


Fig 1: TIC A-Leaf extract of hydrocolyte verticillate D/data. ms

3. Results

3.1 Essential oil components found in *Hydrocotyle verticillata* leaf

Tables 1.1a, 1.1b and 1.1c shows the essential oils present in *Hydrocotyle verticillata* leaf. The demographics of the identified components from the combined GC-MS protocol are presented.

The components with the highest percent abundance in a downward order were Estragole [66.85%], 3.beta., 17. Beta.-dihydroxyestr-4-ene [10.89%], 1,3,7-octatriene, 3,7-dimethyl [9.70%], and so forth in that order.

Table 1.1a: Essential oils constituents present in *Hydrocotyle verticillata* leaf

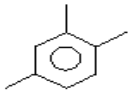
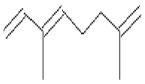
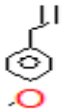
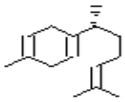
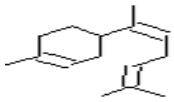
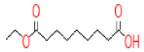
Name of compound	Retention time (RT) (Minutes)	Molecular weight (g/mol)	Peak Area (%)	Molecular Structure
Benzene, 1,2,4-trimethyl-	3.671	120.19	1.702	
1,3,7-Octatriene, 3,7-dimethyl	5.284	136.23	9.699	
Estragole	66.85	148.20	66.847	
(R)-1-Methyl-4-(6-methylhept-5-en- 2-yl)cyclohexa-1,4-diene	9.930	204.35	1.767	
cis-.alpha.-Bisabolene	11.321	204.35	4.784	
Pentadecanal-	13.375	226.40	3.237	
Azelaic acid, monoethyl ester	11.887	216.27	2.25%	

Table 1.1b: Essential oils constituents present in *Hydrocotyle verticillata* leaf

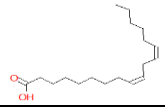
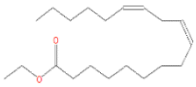
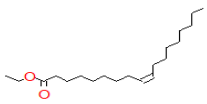
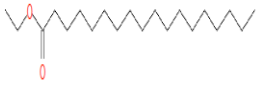
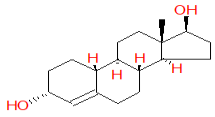
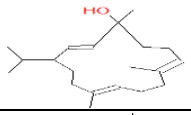
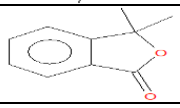
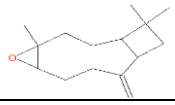
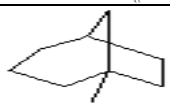
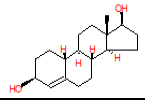
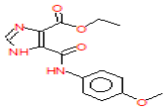
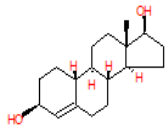
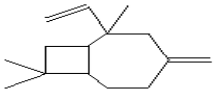
Name of compound	Retention time (RT) (Minutes)	Molecular weight (g/mol)	Peak Area (%)	Molecular Structure
n-Hexadecanoic acid	14.854	256.40	3.45%	
Hexadecanoic acid, ethyl ester	15.159	284.48	7.49%	
9,12-Octadecadienoic acid (Z,Z)-	16.216	280.40	2.93%	
9-Octadecenoic acid	16.287	282.50	3.41%	
Linoleic acid ethyl ester	16.473	310.51	6.90%	
Ethyl Oleate	16.540	310.51	5.65%	
Octadecanoic acid, ethyl ester	16.763	312.50	3.05%	
3.alpha.,17. beta.-dihydroxyestr-4- ene	18.268	276.21	7.06%	

Table 1.1c: Essential oils constituents present in *Hydrocotyle verticillata* leaf

Name of compound	Retention time (RT) (Minutes)	Molecular weight (g/mol)	Peak Area (%)	Molecular Structure
Thunbergol	18.749	290.50	3.13%	
1(3H)-Isobenzofuranone, 3,3-dimethyl-	19.163	162.18	4.21%	
Caryophyllene oxide	19.340	220.35	5.29%	
1-Methylbicyclo [3.2.1] octane	19.616	124.22	7.28%	
3.beta.,17. beta.-dihydroxyestr-4-ene	19.711	276.41	10.89%	
5-(4-Methoxy-phenylcarbamoyl)-1H-imidazole-4-carboxylic acid, ethyl ester	19.987	524.60	3.62%	
3.beta.,17. beta.-dihydroxyestr-4-ene	20.044	276.41	2.91%	
Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	20.297	204.35	0.31%	

4. Discussion of findings

Herbal medicine with their known curative potencies, easy availability, natural way of healing, with minimal toxicity has encouraged the huge premium placed on these products. It is instructive to note that these new leaning on these phytonutrients could be highly related to the established pharmacokinetics, medical properties, and their beneficial effects in many disease conditions [8, 12-13]. That could be argued that the characterization of the essential oils constituents present in *Hydrocotyle verticillata* leaf from our local environment may be of great benefits. Of course, the discovery of any possible beneficial effects of the essential oils in *Hydrocotyle verticillata* pay present it as possible ready sources of natural, safer, readily available and cheaper source of ethno pharmacologic remedies for the mammalian living system.

The current study identified beneficial essential oils in the extract of *Hydrocotyle verticillata* leaf such as linoleic acid ethyl ester, Hexadecanoic acid, ethyl ester, 1-Methylbicyclo [3.2.1] octane, and phytol. Other identified essential oil components inherent in *Hydrocotyle verticillata* includes gamma.Sitosterol as well as Diazo-progesterone. It is a documented evidence that majority of the isolated compounds identified in the essential oil of *H. verticillata* have exerted their effect through many ways particularly those involved in cell cycle regulation and programmed cell death among others [8]. They have been reported to be possess anti-proliferation disorders and inflammation reactions involving reactive species [ROS/RNS] respectively. Other known role includes modulation of damaged DNA [7-8], MAP-Kinase activation route, as well as redox cycling actions involving reducing equivalents have been noted [15]. It is a known fact that linoleic acid ethyl ester possess an excellent detoxification features while other metabolites such as hexadecanoic acid ethyl- ester has been documented to exhibit anti-inflammatory tendencies [15]. A known metabolite with strong effect on viruses and cancer progression were also identified. These were noted to be a member of the cycloalkane family known as 1-Methylbiclo [3.2.1] octane. These compounds were also found to reduce cholesterol as well as its action as an immune-stimulatory agent respectively [8]. Bekinbo *et al*., [16] made a similar submission. The anti-microbial, anti-necrotic and the action on the kidney have equally been noted of the phytol which is a component of the phytol group of compounds [7, 8]. The programmed cell death enhancing action of Diazo-progesterone and gamma. –Sitosterol on some cell lines particularly the HL-60 cells were identified [8]. This submission is in synergy with the work of Khatun *et al*., [7]. These isolated components were known to take part in Bcl-2 enhancing action where they are implicated in influencing apoptotic pathway. A similar work by Kabeel *et al*., [14], were of the view that these isolated compounds were known to act on cancer cell lines by blocking and attenuating the progression of the cancer cell lines through their anti-carcinogenic and anti-mutagenic features. They equally act to suppress leukemia proliferation by the action of induction and enhancement of cell death pathway [8, 14].

5. Conclusions

The essential oils present in *Hydrocotyle verticillata* leaf are abundant in estragole, bis (2-ethylhexyl) phthalate, gamma.-Sitosterol, 9-Octadecenoic acid (Z)-, methyl ester. These have shown to possess anti-inflammatory, anti-oxidant, anti-cancer and neuro-stimulatory properties among others.

6. Conflict of Interest
The authors asserts that there is no conflicts of interest.

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