

# Nutrient and Essential Oil Compositions of *Heterotis rotundifolia* Leaves

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**Abstract:** This study evaluated the nutrient and essential oil compositions of *Heterotis rotundifolia* leaves. The leaves were carefully sorted and air-dried for 14 days at room temperature (20-25°C). The dried leaves were ground into powder using a grinding mill. Proximate composition, vitamins and physicochemical properties were analyzed using AOAC method while fatty acid and essential oil compositions were characterized using GC-MS. The proximate composition of the leaves showed higher carbohydrate content (80.97±0.03%) and moderate protein content (9.9±0.06%); while lipid (3.13±0.01%), fibre (1.30±0.01%), moisture (2.90±0.03%) and ash (1.89±0.02%) contents were low. Physicochemical properties of the oil revealed a high saponification value (238.43±0.84mg/KOH) and peroxide value (47.20±0.38mEq/kg). Fatty acid composition shows 83.69% saturated fatty acid with stearic acid (22.69±0.02%), myristic acid (18.54±0.01%) and palmitic acid (15.48±0.02%) as predominant fatty acids. Unsaturated fatty acid composition was 9.72% with oleic acid (9.49±0.04%) and linolenic acid (0.23±0.01%) as predominant fatty acids. Vitamin composition also revealed higher concentrations of vitamins C (695.57±0.20mg/kg), B<sub>6</sub> (70.33±0.88mg/kg), A (61.67±0.02mg/kg) and D (26.08±0.06mg/kg). Essential oil composition revealed a total of 24 compounds which include; long chain fatty acid, fatty acid methyl esters, hydrocarbons and alcoholic compound amongst which Neophytadiene (14.427%), n-Hexadecanoic acid (14.148%) and squalene (11.258%) were present in substantial amount. The proximate and essential oil compositions, vitamin content and physicochemical characteristics suggest that *H. rotundifolia* leaves have potential nutritional and medicinal value to man and animals.

**Keywords:** *Heterotis rotundifolia*, Leaves, Proximate Composition, Vitamins, Fatty Acid, Essential Oils

## 1. Introduction

All over the world, the focus on plant research has increased greatly in recent times and enormous observations on the potential of medicinal plants employed in traditional systems have been discovered [1]. Plants have served as the basis of sophisticated traditional medicine system for thousands of years in countries such as China and India. World Health Organization has described traditional medicine as one of the surest means to achieve total health care coverage of the world population [2]. Literature survey shows that the therapeutic efficacy of herbal plant is on the basis of proximate and elemental compositions. Proximate analyses of medicinal plants play a vital role in evaluating

their nutritional significance [3, 4]. To better understand the worth of various medicinal plant species which are also used as food along with their therapeutic benefits, determination of their nutritional significance is therefore essential. World Health Organization (2005) [5] therefore emphasizes the importance and need of determining proximate and micronutrients composition as well as phytochemical constituents in establishing standardization of the medicinal plants and herbal products [6].

Essential oils are volatile, lipid-soluble secondary metabolites of aromatic plants, synthesized from all parts of the plant and are characterized by their strong odor. They are used in the cosmetics and pharmaceutical industries for their fragrances, taste, antibacterial, antifungal, antiviral, analgesic, sedative, anti-inflammatory, spasmolytic and local

anesthetic properties as well as insecticide activity [7]. Since volatile oils are known to contribute to the therapeutic properties of medicinal plants, it is therefore imperative to identify the various essential oils present in the plants and scientifically evaluate these medicinal plants.

*Heterotis rotundifolia* is a perennial decumbent herb that belongs to the *Melastomataceae* family and widely found in tropical West Africa. Wagner *et al.* (1990) [8] identified *H. rotundifolia* as pink lady or Spanish shawl in English. In Nigeria, different tribes identify it with different names. It is called 'Nkpisi-nku' in Igbo, 'Awede' in Yoruba, 'Ebafo' in Benin and 'Akpalijhie' in Ikwerre. *Heterotis rotundifolia* is a versatile slender creeping herb with ascending stems, rooting at the nodes and can grow up to 40cm long [9]. The plant can be propagated either by seeds or vegetation [10]. It can be found in moist or damp places in forests (under shade of trees), along streams, roadsides growing as weed, disturbed areas, in waste spaces, bottom of sandy depressions [11] and sometimes on rocks or creeping and climbing among boulders and in open woodland. In Nigeria, the plant is used mainly for the treatment of rheumatism and painful swellings and the leaves decoction is used to relieve stomach ache, diarrhea, dysentery, cough, conjunctivitis, circulatory problems and venereal diseases [8].



Figure 1. *Heterotis rotundifolia* plant showing the leaves, flower and stem.

## 2. Materials and Methods

### 2.1. Plant Collection and Identification

Freshly harvested leaves of *Heterotis rotundifolia* were obtained from the University of Port Harcourt environment. Samples were identified and authenticated by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt Herbarium with voucher number UPH/V/1323.

### 2.2. Sample Preparation

The detached leaves from the collected plant materials

were carefully sorted and air-dried at room temperature for 14 days. The dried leaves were ground into powder using a grinding mill and sieved using 100mm mesh.

### 2.3. Determination of Proximate Composition

Analysis of the proximate composition (moisture, ash, protein, fat, fibre and carbohydrate content) of the *H. rotundifolia* leaves were performed using Association of Official Analytical Chemists (AOAC, 1984) [12] methods. Kjeldahl apparatus was used for the estimation of nitrogen content and protein content was calculated as  $N \times 6.25$  while crude fat was done using Soxhlet extraction method.

The carbohydrate content was determined by difference [100 - (Protein +Fats +moisture +ash+ fibre)].

### 2.4. Determination of Physicochemical Parameters

Physicochemical parameters of the leaves of *Heterotis rotundifolia* such as refractive index, density, viscosity, acid value, peroxide value, iodine value, saponification value, free fatty acid and thiobarbituric acid were estimated by the method of AOAC (1990) [13].

### 2.5. Determination of Fatty acid Composition

The fatty acid composition was determined using GC/MS. Ten (10) gram of the powdered sample was subjected to soxhlet extraction with 300ml of n-hexane for 24 hours; the solvent was evaporated to dryness using a rotary evaporator at 40°C. Sample was prepared by dissolving 1ml of filtered residue in 50ml chloroform and evaporated at room temperature. One milliliter of reagent (benzene and methanol 20:55vol%) was added and heated for 30mins at 40°C. Organic sample was extracted with hexane and water with a vigorous shake. About half of the top phase of hexane was taken for GC/MS analysis following the method of Patel *et al.* (2017) [14]

### 2.6. Determination of Vitamin Contents

Vitamin A was estimated by Bayfield and Cole (1980) [15]; while vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub> and B<sub>12</sub> were determined by spectrophotometric method. Ascorbic acid was determined by spectrophotometric method as described by Roe and Keuther (1943) [16] and vitamin E by Emmeric-Engel method as described by Rosenberg (1992) [17].

### 2.7. Determination of Essential oil Components

Powdered sample weighing 10g was measured and extracted in 20ml of dichloromethane and filtered for three consecutive times into a quartz beaker. The combined aliquots were concentrated on a steam bath to about 5ml and purified through a Pasteur pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air-dried to about 2ml for GC analysis. GC/MS analysis [14] was performed on an Agilent Technologies equipment (model 7890A) interfaced with Mass Selective Detector model 5975C. Using n-alkanes as a reference range, the retention indices for all compounds

were recorded according to the method described by Dool and Kratz (1963) [18]. Identification of the compounds was achieved by comparing their mass spectra with some reference compounds contained in the National Institute of Standards and Technology (NIST) libraries [19] and those described by Adams (1995) [20].

## 2.8. Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 22 was used to process and analyze the data obtained. Values were expressed as means  $\pm$  standard error mean (SEM).

## 3. Results and Discussion

**Table 1.** Proximate composition of *Heterotis rotundifolia* leaves.

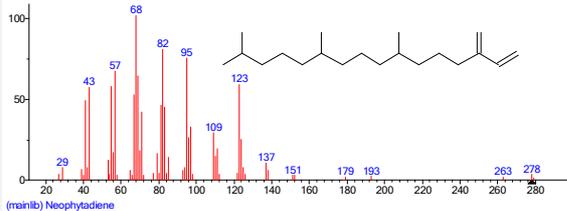
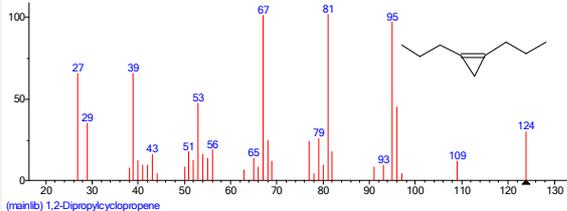
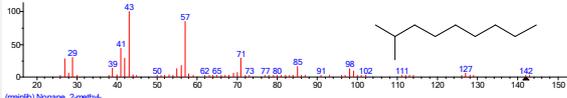
Parameters	Concentration (%)
Ash	1.89 $\pm$ 0.02
Moisture	2.90 $\pm$ 0.03
Protein	9.90 $\pm$ 0.06
Fibre	1.30 $\pm$ 0.01
Fat	3.13 $\pm$ 0.01
Carbohydrate	80.88 $\pm$ 0.03

Values are reported as mean  $\pm$  standard error mean (SEM) of triplicates determination.

**Table 2.** Physicochemical properties of *Heterotis rotundifolia* leaves.

Parameters	Concentration
Saponification value (mg/KOH)	238.43 $\pm$ 0.84
Peroxide value (mEq/kg)	47.20 $\pm$ 0.38
Acid value (%)	4.22 $\pm$ 0.05
Free fatty acid (%)	2.11 $\pm$ 0.05
Iodine value	32.99 $\pm$ 0.34
Refractive index	1.41 $\pm$ 0.03
Viscosity (Pa.S)	1.61 $\pm$ 0.04
Density (g/ml)	0.92 $\pm$ 0.01

**Table 5.** Chemical composition of essential oil of air-dried *Heterotis rotundifolia* leaves.

Compound	Retention Time (min)	Percentage composition	Molecular formula	Molecular weight	Structure
Neophytadiene	17.987	14.427	C <sub>20</sub> H <sub>38</sub>	278.5157	
1, 2-Dipropyl Cyclopropene	18.382	4.062	C <sub>9</sub> H <sub>16</sub>	124.2230	
1, 8-Nonadiene, 2, 8-dimethyl-	18.662	4.227	C <sub>11</sub> H <sub>20</sub>	152.2765	

Parameters	Concentration
Thiobarbituric acid (mg/kg)	2.48 $\pm$ 0.02

Values are reported as mean  $\pm$  standard error mean (SEM) of triplicates determination.

**Table 3.** Fatty acid composition of *Heterotis rotundifolia* leaves.

Parameters	% composition
C12 Lauric acid	14.25 $\pm$ 0.02
C14 myristic acid	18.54 $\pm$ 0.01
C16 palmitic acid	15.48 $\pm$ 0.02
C18 stearic acid	22.69 $\pm$ 0.02
C18:1 Oleic acid	9.49 $\pm$ 0.043
C18:2 Linoleic acid	ND
C18:3 Linolenic acid	0.23 $\pm$ 0.00081
C20 Arachidic acid	0.0028 $\pm$ 0.00007
C20:4 Arachidonic acid	12.73 $\pm$ 0.02
Total FA	93.41
Total SFA	83.69
Total USFA	9.72

ND= Not Detected.

**Table 4.** Vitamin contents of *Heterotis rotundifolia* leaves.

Parameters	Concentration (mg/kg)
Vitamin A	61.67 $\pm$ 0.02
Vitamin B1	0.27 $\pm$ 0.02
Vitamin B2	0.59 $\pm$ 0.01
Vitamin B3	0.28 $\pm$ 0.01
Vitamin B6	70.33 $\pm$ 0.88
Vitamin B12	0.21 $\pm$ 0.01
Vitamin C	695.57 $\pm$ 0.23
Vitamin D	26.08 $\pm$ 0.06
Vitamin E	5.45 $\pm$ 0.03

Values are reported as mean  $\pm$  standard error mean (SEM) of triplicates determination.

Compound	Retention Time (min)	Percentage composition	Molecular formula	Molecular weight	Structure
Hexadecanoic acid, methyl ester	19.497	1.921	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.4507	
n-Hexadecanoic acid	20.396	14.148	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4241	
9, 12-Octadeca dienoic acid, methylester, (E,E)-	22.278	0.589	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.4721	
n-Hexadecanoic acid	22.690	0.641	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4241	
9, 12-Octadecadienoic acid (Z,Z)-	23.245	5.913	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.4455	
Eicosanoic acid, methyl ester	25.797	0.467	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.5570	
Bis (2-ethylhexyl) phthalate	28.481	7.633	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.564	
Dotriacontane, 1-iodo-	29.648	1.029	C <sub>32</sub> H <sub>65</sub> I	576.7630	

Compound	Retention Time (min)	Percentage composition	Molecular formula	Molecular weight	Structure
Eicosane	30.512	0.729	C <sub>20</sub> H <sub>42</sub>	282.5475	
Squalene	30.867	11.258	C <sub>30</sub> H <sub>50</sub>	410.718	
.alpha.-Tocospiro B	31.245	1.433	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	462.7049	
Nonacosane	31.308	3.767	C <sub>29</sub> H <sub>60</sub>	408.7867	
.alpha.-Tocospiro B	31.416	3.118	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	462.7049	
Eicosane	32.069	1.200	C <sub>20</sub> H <sub>42</sub>	282.5475	
N-Methyl-1-adamantaneacetamide	32.721	0.564	C <sub>13</sub> H <sub>21</sub> NO	207.317	
Eicosane	32.778	5.257	C <sub>20</sub> H <sub>42</sub>	282.5475	

Compound	Retention Time (min)	Percentage composition	Molecular formula	Molecular weight	Structure
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl-	33.150	1.100	C <sub>16</sub> H <sub>50</sub> O <sub>7</sub> Si <sub>8</sub>	579.248	
Vitamin E	33.413	6.079	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.7100	
Eicosane	34.226	2.377	C <sub>20</sub> H <sub>42</sub>	282.5475	
Campesterol	34.415	1.605	C <sub>28</sub> H <sub>48</sub> O	400.691	
Stigmasterol	34.644	6.456	C <sub>29</sub> H <sub>48</sub> O	412.702	

The results of the proximate composition of *H. rotundifolia* leaves (Table 1) revealed the following composition; 1.89% ash, 2.90% moisture, 1.30% fibre, 3.13% fat, 9.9% protein and 80.97% carbohydrate contents. In this study, the proximate composition of *H. rotundifolia* leaves revealed a high concentration of carbohydrate (80.88%) suggesting that it can be an important source of energy for the body. The high carbohydrate value of the leaves is similar to the reports by Ayevbuomwan *et al.* (2017) [21] for *H. rotundifolia* leaves (80.36%) and Asibey-Berko and Tayle (1999) [22] for *Tribulus terrestris* (53.67%). Reason for high level of carbohydrate in the leaf sample may be attributed to the fact that during photosynthesis, glucose produced is stored as starch in the leaves. Although the accumulation of non-structural carbohydrate in leaves represses photosynthesis. However, the extent of repression should be different between sink leaves (sugar consumers) and source leaves (sugar exporters) [23]. The ash content of the leaves (1.89%) was lower than the value (3.02%) reported by Ayevbuomwan *et al.* (2017) [21] suggesting low mineral concentration. The moisture and fibre contents (2.90% and 1.30%) of *H. rotundifolia* leaves were found to be lower compared with 6.83% and 5.42% respectively as

reported by Ayevbuomwan *et al.*, 2017 [21] and in dried *Talinum triangulare* (waterleaf) leaves (13.4% and 4.0%) as reported by Orhuamen *et al.* (2012) [24]. The percentage moisture of *H. rotundifolia* leaves is similar to the result (0.76%) obtained by Orhuamen *et al.* (2012) [24] for dried leaves of *Telfairia occidentalis* (fluted pumpkin), suggesting that the low moisture of *H. rotundifolia* leaves might retard microbial growth and reduce spoilage before being utilized or processed. Crude fibre in food or plant shows non-digestible carbohydrate and lignin level. This low fibre content can be regarded as suitable because it promotes glucose and fat absorption. Although, crude fibre enhances digestibility but a high level can cause intestinal irritation as well as lower digestibility and nutrient usage. Crude fibre comprises majorly of cellulose and a little lignin that is indigestible in humans [25]. The crude fat and protein contents of the leaves of *H. rotundifolia* were higher compared with 1.75% and 2.62% respectively as reported by Ayevbuomwan *et al.* (2017) [21]. The substantial quantity of protein content (9.9%) of *H. rotundifolia* leaves suggests that it can be an indispensable component vital for the sustenance and repair of worn out tissues, supply of energy and adequate amount of required amino acids [26]. These variations in proximate

compositions of *H. rotundifolia* leaves with previous reports may probably be caused by soil composition, climatic conditions and geographical location.

The physicochemical properties of *H. rotundifolia* leaves (Table 2) revealed the following results; saponification value (238.43mg/KOH), Peroxide value (47.20mEq/kg), Acid value (4.22%), Free fatty acid (2.11%), Iodine value (32.99), Refractive index (1.41), viscosity (1.61Pa.S), Density (0.92g/ml) and Thiobarbituric acid (2.48mg/kg).

The physicochemical properties revealed that the peroxide value of *H. rotundifolia* was higher compared with *Duranta repens* (yellow bush) leaves as reported by Agomuo et al. (2017) [27]. According to Aremu et al. (2015) [28], peroxide value has been recognized as an index of lipid oxidation and high quantities of peroxide value indicates high rancidity level. WHO (1995) [29] identified the permissible maximum peroxide value for oils as 10meq of O<sub>2</sub>/kg. This therefore suggests that *H. rotundifolia* leaves oil could be vulnerable to autoxidation. The Iodine value (32.99gI<sub>2</sub>/100g) of *H. rotundifolia* leaves is lower than *D. repens* leaves oil (72.65g I<sub>2</sub>/100g). Iodine value determines fatty acid unsaturation level. Iodine value of *H. rotundifolia* leaves can be categorized as non drying oil because its iodine value was found to be below 100gI<sub>2</sub>/100g. The saponification value ((238.43mg/KOH) of *H. rotundifolia* leaves was higher compared with *D. repens* (166.93mg/KOH) but comparable to palm kernel oil at 195-205mg/KOH [28]. High saponification value is recognized as an index of increased oil quality since it specifies the existence of high proportion of low molecular weight fatty acids in oils. Acid value also gives an indication of oil quality, stability and edibility [30]. The acid value of the leaves was seen to be lower than *D. repens* but similar to *Moringa oleifera* plant as reported by Belay and Sisay (2014) [31]. This implies that the oil of *H. rotundifolia* leaves might account for long term stability and protection against rancidity and peroxidation. Also it can be fit for cooking and livestock feeding [28]. Refractive index of the oil obtained in this study did not fall within the acceptable range (1.4677-1.4707) for virgin and refined oil as stated by Codex Standards for fats and oils from vegetable/plant [32].

The fatty acid composition of *H. rotundifolia* leaves presented in Table 3 showed the presence of eight fatty acids with stearic acid (22.69±0.02%) having the highest concentration followed by myristic acid (18.54±0.01%), palmitic acid (15.48±0.02%), lauric acid (14.25±0.02) and arachidonic acid (12.73±0.02%). The least fatty acid identified was arachidic acid (0.0028±0.00%). *Heterotis rotundifolia* leaves are composed more of saturated fatty acid having a total value of 83.69% and unsaturated fatty acid having 9.72%. Arachidonic acid recorded the highest value in the category of unsaturated fatty acids; although the amounts of unsaturated fatty acids are generally low. However, unsaturated fatty acids are fatty acids with more double bonds and are easily attacked by free radicals. This shows that *H. rotundifolia* leaves with high levels of saturated fatty acids may trigger cardiovascular problems due to increase in

blood cholesterol [33].

Results of the vitamin composition presented on Table 4 revealed the presence of Vitamins A (61.67±0.02mg/kg), B<sub>1</sub> (0.27±0.02mg/kg), B<sub>2</sub> (0.59±0.01mg/kg), B<sub>3</sub> (0.28±0.01mg/kg), B<sub>6</sub> (70.33±0.08mg/kg), B<sub>12</sub> (0.21±0.01mg/kg), C (695.57±0.23mg/kg), D (26.08±0.06mg/kg) and E (5.45±0.03mg/kg). The results shows that the leaves are relatively higher in Vitamins C, B<sub>6</sub>, B<sub>2</sub>, A and D when compared to the report by Offor, (2015) [34]. Vitamin C as an antioxidant, performs major functions which includes; synthesis of collagen, sustenance of normal connective tissue, angiogenesis and wound healing and also aid dietary iron absorption from the intestine. Vitamin A performs various functions including growth, vision, reproduction and maintenance of epithelial tissues and mucous membrane. Vitamin B<sub>6</sub> participates in erythropoietin production, metabolism of carbohydrates, liver detoxification, nervous system health and brain function. Vitamin D stimulates the movement of calcium and phosphate from the bone. It also participates in white blood cells maturation [35]. The appreciable amount of vitamins in this leaf sample shows that it could serve as viable nutritional supplements.

The essential oil composition of *Heterotis rotundifolia* leaves revealed the existence of twenty-four compounds which amounted to 100% of the total essential oil constituents. The identified compounds of the dried leaves of *H. rotundifolia*, their retention times, percentage composition, molecular formula, molecular weight and their structures are given on Table 5.

The identified essential oils possess numerous therapeutic activities biologically predominant in pharmaceutical companies. From this study, Neophytadiene (14.427%) was detected as the major abundant compound on GC/MS analysis. The percentage composition of Neophytadiene in *H. rotundifolia* leaves was found to be lower than the value (35.8%) reported by Adeosun et al. (2017) [36] for *Jatropha curcas* leaves. Neophytadiene, a hydrocarbon compound has been detailed to have antipyretic, anti-inflammatory, antimicrobial analgesic and antioxidant biological properties [14]. The second most abundant compound detected was n-Hexadecanoic acid. N-Hexadecanoic acid is a saturated fatty acid reported by Adeoye-Isijola et al. (2018) [37] to possess antioxidant, antibacterial, nematicide, anti-inflammatory, hypocholesterolemic, pesticide, lubricant, anti-androgenic, antitumor, flavour, cancer preventive, immunostimulant, chemo preventive, hemolytic 5- $\alpha$  reductase inhibitor and lipo-oxygenase inhibitor. The percent composition of Squalene in the study is lower than 52.74% reported by Ammal and Bai (2013) [38] for *Heliotropium indicum* (India heliotrope) leaf. Squalene, the third most abundant compound identified is a triterpene saturated fatty acid that possesses antibacterial, antioxidant, pesticide, immunostimulant, anticancer, perfumery, antitumor, sunscreen and cancer preventive properties [39]. Stigmasterol is a precursor of progesterone (steroid compound) that contains antioxidant, hypoglycaemic and thyroid inhibiting properties, anticancer, antiasthma, antiarthritic, antidiuretic,

anti-inflammatory and antimicrobial properties [14]. The compound; 9, 12-Octadecadienoic acid is a linoleic fatty acid used as antihistaminic, antiacne, hepatoprotective, antiarthritic, anti-inflammatory, anti-androgenic, hypocholesterolemic, anticoronary and 5-alpha-reductase inhibitor as reported by Bihana *et al.* (2018) [39].

## 4. Conclusion

The result of the present investigation revealed that *Heterotis rotundifolia* leaves are rich sources of carbohydrate, proteins, vitamins and essential oils. The high saponification value indicates that the leaves will be stable to rancidity. Essential oil composition also revealed that the leaves of *H. rotundifolia* are rich in bioactive components that possess wide range of biological activities such as anti-cancer, anti-inflammatory, anti-pyretic, analgesic, antioxidant, antimicrobial amongst others.

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