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BIOACTIVE PROFILING, ANTIOXIDANT, ANTIBACTERIAL AND CHEMICAL ANALYSIS OF ESSENTIAL OILS FROM THE LEAVES OF *Duabanga grandiflora* IN RAJASTHAN INDIA

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Abstract. *Duabanga grandiflora* is one natures overlooked medicinal plant with a cocktail of bioactive compounds which goes far beyond nutrition capable of healing, restoring and protecting good health in both human and animals. Bioactive profiling, antioxidant, antibacterial and chemical analysis of essential oils from the leaves of *Duabanga grandiflora* in Rajasthan India showed that it contained 38 bioactive compounds with therapeutic properties during GC-MS analysis with squalene (11.66%), neophytadiene (8.65%) hexadecanoic acid (8.12%), 2-methyloctacosane (7.18%), phytol (6.61%), butyl tetratriacontyl ether (6.77%), hexadecanoic acid, ethyl ester (6.32%), 1,8-cineole (5.62%), octadecanoic acid (5.11%) and chloromethyl 7-chlorododecanoate (4.09%) as predominant compounds. The oil also shows strong antioxidant profile by neutralizing the activities of free radicals and strengthen the immune system. *Duabanga grandiflora* oil proved it efficacy against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Shigella flexineri* and *Salmonella typhi*. thus fueling beneficial bacteria which lowers inflammation and reduce the risk of diseases. The leaf of *Duabanga grandiflora* also contained moisture (13.42%), crude protein (15.68%), crude fibre (9.91%), ether extract (0.69%), ash (12.07%) and energy (1520.3 Kcal/kg) and important minerals; potassium had the highest concentration of 909.61 mg/100g followed by calcium (305.24 mg/100g), phosphorus (164.90 mg/100g), sodium (100.3 mg/100g), magnesium (87.32 mg/100g), iron (10.96 mg/100g), zinc (4.51 mg/100g), copper (1.57 mg/100g), nickel (0.25 mg/100g) and cobalt (0.10 mg/100g). In conclusion, *Duabanga grandiflora* is truly a pharmacy in disguise and can be used to combat antimicrobial resistance and promote food security.

Keywords: *Antimicrobial, inflammation, phytochemicals, Duabanga grandiflora, food security*.

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1. Introduction

According to the World Health Organization in 2014, categorized medicinal plant is any plant which, in one or more of its organs, contains substances (phytochemicals or bioactive compounds) that can be used for therapeutic or pharmacological purposes or which are precursors for chemo-pharmaceutical semi-synthesis. Such a plant will have its parts including, stem bark, buds, roots, seeds, flowers, rhizomes amongst others employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active (Alagbe, 2021).

Duabanga grandiflora also Bandorhula is an evergreen medicinal plant belonging to the family Lythraceae (Ribeiro *et al*., 2000; Saha *et al*., 2021). The plant is a pharmacy in disguise and its one of natures overlooked medicines native to Asia and some parts of

Africa. The leaves, seeds, fruits and flowers carry unique phytochemicals that have been used by traditional healers to treat infections, soothe inflammation and support recovery from chronic diseases (Debnath *et al*., 2021). Traditional medicine use *Duabanga grandiflora* poultices for wounds, ulcers and microbial infections (Chu, 2014; Tsukiyama *et al*., 2010). Research published by (Dabnath *et al*., 2020), has shown that *Duabanga grandiflora* leaf extract can inhibit hexokinase, slowing glucose metabolism and help to stabilize blood sugar level (Sadhu *et al*., 2003; Ayoola *et al*., 2008). The leaves also deliver powerful antioxidant protection neutralizing free radicals helping to protect cellular damage linked to cardiovascular disease and neuro-degeneration (Akhter *et al*., 2019; Kaweetripob *et al*., 2012).

Leaf infusions from the plant also has the potential to regulate lipid metabolism and reduce fat accumulation (Asif, 2016). They also show strong antibacterial actions against *Escherichia coli*, *Salmonella spp* and *Streptococcus spp*. Recent studies have shown that aqueous, methanolic and ethanolic extract from the leaves of *Duabanga grandiflora* contains immuno-modulatory compounds that help to balance immune activity and stimulating it when body defenses are low and calming it when it is over reactive (Bushra *et al*., 2010). This dual action helps the body to respond to threat against infections (Altman *et al*., 2015). *Duabanga grandiflora* has a cocktail of anti-inflammatory compounds with a long chain fatty alcohol that improves mitochondria function that may reduce blood cholesterol level (Cordovilla-Guardia *et al*., 2019). These compounds have act as liver protectors, metabolic regulators reducing inflammation and restoring enzymatic balance in the gut (Datta *et al*., 2004).

Studies have shown that location, age of plant, species, parts of plants and processing method could influence the chemical composition of medicinal plants (Hernandez & Alagbe, 2025). However, there is a little or no information on the bioactive and chemical compounds of *Duabanga grandiflora* oil. This research is timely, as it will help to address the increasing global challenges on antimicrobial resistance, could also promote food security and lead to the discovery of novel medicinal drugs for human beings and animals.

2. Materials and methods

2.1. Collection and identification of Duabanga grandiflora leaves

Fresh leaves of *Duabanga grandiflora* was collected from the botanical garden of Gandhi College of Agriculture, Rajasthan India. The collected leaves were sent to the Taxonomy Department, Sumitra Research Institute, Gujarat for proper authentication before it was provided a reference number (HM/08A/202). The leaves were further sorted, washed in water followed by distilled water to remove dirt and spread on a plastic sieve for 30 minutes in order to allow water to drain.

2.2. Extraction of Duabanga grandiflora oil

Extraction of oil from the leaves of *Duabanga grandiflora* was carried out using steam distillation technique in an all-glass Clevenger-apparatus for 4 hours using a 5 L quick fit round bottom flask. The oils were dried in desiccators containing anhydrous sodium sulphate for 24 hours and then stored in airtight vials in a refrigerator at 4 ℃.

2.3. Analysis of bioactive compounds in Duabanga grandiflora oil

Bioactive compounds in *Duabanga grandiflora* oil was analyzed on an Agilent Model 7890A Gas Chromatography-Mass spectrometry equipped with a HP-5ms fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Analytical conditions were adjusted to: Oven temperature: 55℃, with 5 minutes' initial hold and then to 300℃ at 5℃/min, with final hold time of 20 minutes; helium was used as carrier gas at a flow rate of 1 mL/min. The mass spectrometry compartment was operated with ionization voltage 70 eV and ion source temperature, 280℃. Relative percentage amounts of the essential oil components were evaluated from the total peak area by Agilent GC-MS software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with reference compounds from the Library of National Institute of Standard and Technology (2011) database.

2.4. Assessment of antioxidant activities

DPPH free radical scavenging assay

The free radical scavenging capacity of the essential oil from *Duabanga grandiflora* was determined using the 1,1-diphenyl-2- picrylhydrazyl (DPPH) procedures published by Alhage et al. (2018). Briefly, 1mL of DPPH solution was added to 1 mL of the essential *Duabanga grandiflora* oils at different concentrations (25, 50, 75, 100 μg/mL). Mixture was shaken and kept in the dark for 30 min and Vitamin C was used as used as positive control after which the absorbance was read at 517 nm in a UV Spectrophotometer. Lower absorbance of the reaction mixture suggested higher free radical scavenging activity and the test was done in triplicates. The % DPPH scavenging effect was estimated by plotting inhibition % against concentration to obtain the IC50.

$$\% \text{ Inhibition} = \frac{G_1 \text{ control} - G_2 \text{ sample}}{G_1 \text{ control}} \times 100$$

where, G_1 control refers to the absorbance of the control (DPPH solution without sample) and G_2 sample refers to the absorbance of the test sample (DPPH solution with test sample) at 517 nm.

2.5. Lipid peroxidation

Lipid peroxidation assay was carried out according to the procedures outlined by Molefi and Pillai (2021). In this assay, 20 μL of *Duabanga grandiflora* oil at different concentrations of 25, 50, 75 and 100 μg/mL (standard solution) followed by the addition of 40 μL (1,1,3,3- tetramethoxypropane) and 40 μL of 20 mM phosphate buffer (pH 7.0) in test tubes on an ice bath. In each tube, 50 μL of 3% sodium dodecyl sulfate, 200 μL of 0.1 N Hydrochloric acid, 30 μL of 10% phosphotungstic acid and 100 μL of 0.7% of 2-thiobarbituric acid (TBA) were further added and boiled in water bath at a temperature of 100°C for 30 min. The mixture was passed through U/V Spectrophotometer and read at 515 nm using Ascorbic acid as positive control.

2.6. Ferric reducing antioxidant power analysis

Ferric reducing antioxidant power analysis was carried out according to the procedures published by Olaiya et al. (2016). Gallic acid was used as the control and the absorbance at 700 nm was read using Ultraviolet Visible Spectrophotometer against a blank. The percentage ferric reducing antioxidant power Scavenging effect was calculated as described for the DPPH assay.

2.7. Antibacterial screening

Duabanga grandiflora essential oils were screened for antibacterial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri* and *Salmonella typhi* representing Gram positive and Gram negative bacteria. Microplate Alamar Blue Assay was used to determine susceptibility or resistance of the essential oil to selected bacteria strains. Organisms were cultured in Mueller Hinton broth and inoculums were adjusted to 0.5 McFarland standard. Stock solution of the oil was prepared in DMSO (1:1 concentration). Media was dispensed to all wells. 20 µg/mL of *Duabanga grandiflora* oil was added in the wells, control well do not contain essential oil. The volume of 96-well plate was made up to 200 µL. Finally, $5x10^6$ cell was added in the well including both control and test. The plate was sealed with parafilm and incubated for 18-20 hours. Alamar Blue Dye was dispensed in each well and shaken at 80 RPM in a shaking incubator for 2-3 hours. Plates were covered with foil in shaking incubator. Change in color of Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Absorbance was recorded at 570 nm and 600 nm by the Morten Automated Mico-biolog reader® (Model CVM/08T/KL, China). Ampicillin was used as the reference drug. The experiment was done in triplicate.

2.8. Proximate and mineral analysis of Duabanga grandiflora leaf

Proximate composition of *Duabanga grandiflora* leaf was carried out according to Association of Analytical Chemist Procedure of 2012. Analysis of minerals was done using Comix® Atomic Absorption Spectrophotometer (Model HN-09C-008, Belgium). Machine was operated according to standard operating procedure on the manufacturers manual.

3. Result and discussion

Bioactive profiling of *Duabanga grandiflora* oil by GC-MS in Table 1 reveals the presence of 34 compounds representing 92.51%. The predominant compounds were; squalene (11.66%), neophytadiene (8.65%) hexadecanoic acid (8.12%), 2-methyloctacosane (7.18%), phytol (6.61%), butyl tetratriacontyl ether (6.77%), hexadecanoic acid, ethyl ester (6.32%), 1,8-cineole (5.62%), octadecanoic acid (5.11%) and chloromethyl 7-chlorododecanoate (4.09%) which have been previously associated with a pharmacological power house capable of blocking inflammatory pathways, suppressing pain mediators, protect against oxidative stress, skin regeneration and delivers broad-spectrum antimicrobial effects (Alagbe *et al*., 2021). Squalene has also been previously associated with preventing infections and accelerate healing of wounds and ulcer (Bashir *et al*., 2020). Hexahydrofarnesyl acetone (0.92%), 9-tetradecenal (0.29%), trans-nerolidol (0.17%), cis-vaccenic acid (0.12%) were among the minor compounds with potent inhibitors of cancer cell growth working by blocking the energy

supplied inside malignant cells while sparing healthy tissues (Adewale *et al*., 2021). β-caryophyllene epoxide (0.19%), humulane-1,6-dien-3-ol (0.02%), myristic acid, isopropyl ester (0.14%), β-farnesene (0.12%), acoradiene (0.62%), pentadecane (0.18 %) and α-cedrene (0.41%) have strong antimalarial and antioxidant effects (Morris & Alagbe, 2025). Germacrene-D-4-ol (0.67%), sulforous acid, nonyl pentyl ester (0.25%) and β-linalol (0.16%), protects the liver by reducing damage and supporting detoxification (Velu *et al*., 2018; Kujar *et al*., 2010). Its chemistry goes further as α-bergamotene and 10-undecyn-1-ol produces potent immuno-modulatory effects (Rao & Gan, 2014; Singh and Singh, 2008). The synergy in the efficacy of these compounds in animals have shown its capability to protect the heart by lowering blood cholesterol and reducing the risk of neuro-degenerative disease in animals (Singletary, 2020). The outcome obtained in this study confirms the earlier report of (Altman *et al*., 2015). However, Ribeiro et al. (2000) reported the concentration of squalene to be 9.93 % which is less than 11.66% obtained in this study. The little variation in the values could be as a result of different locations in which the samples of medicinal plants were collected, kit used for analysis, processing method as well as age of plants (Bashir *et al*., 2020).

The antioxidant properties of *Duabanga grandiflora* oil is presented in Table 2. *Duabanga grandiflora* oil DPPH (IC50 of 67.88 µg/mL), ferric reducing antioxidant power assay (IC50 of 1344.7 µg/mL) and lipid peroxidation scavenging activity (IC50 of 82.15 µg/mL) compared to standard drug with DPPH (IC50 of 21.90 µg/mL), FRAP (IC50 of 810.6 µg/mL) and lipid peroxidation (IC50 of 27.03 µg/mL). *Duabanga grandiflora* oil used in this study demonstrated higher antioxidant activity for DPPH and Ferric reducing antioxidant power (FRAP) than the standard drug (Ascorbic and gallic acid). The analysis of the essential oil components in this study revealed that the oils were mainly dominated by sesquiterpenes (36.44%) and monoterpenes (38.08%). The high antioxidant activity of *Duabanga grandiflora* oil is due to the presence of phenolic compounds and high concentrations of monoterpene and sesquiterpene hydrocarbons. According to Miguel et al. (2010), there is a correlation between bioactive compounds in essential oils and their antioxidant properties. Antioxidant properties of essential oils could make them utilized as natural antioxidants and also a model for new free radical scavenging medicines in order to prevent chronic diseases (Hernandez & Alagbe, 2025; Morris & Alagbe, 2025).

The result obtained is in agreement with the report of Guimarães (2010) reported discovered antioxidant activity of essential oils from *Lippia sidoides, Alomia fastigiata, Ocotea odorifera, Mikania glauca and Cordia verbenacea* and their bioactive constituents, by the methods of the β-carotene/linoleic acid oxidation system and the reduction of the stable DPPH radical. Similarly, a report by Kadri et al. (2011) have proven that essential oil from Artemisia are excellent potential source of natural antioxidants in foods in order to find possible alternative to synthetic antioxidant and the pharmaceutical industry for the prevention and the treatment of various human diseases. Synergy in bioactive compounds in essential oil may contribute to their antioxidant properties (Tiwari, 2001).

Table 1. Bioactive profiling of *Duabanga grandiflora* oil by GC-MS

Compounds	% Concentration	Retention time (min)
Hexadecanoic acid, ethyl ester	6.32	481
9-Tetradecenal	0.29	498
Octadecanoic acid	5.11	501
Hexahydrofarnesyl acetone	0.92	526
n-Hexadecanoic acid	8.12	547
2-methyloctacosane	7.18	559
Phytol	6.61	592
Neophytadiene	8.65	598
Squalene	11.66	601
Chloromethyl 7-chlorododecanoate	4.09	628
Butyl tetratriacontyl ether	6.77	636
9,12-Octadecadienoic acid, methyl ester	4.02	655
Trans-9-Octadecenoic acid, pentyl ester	2.18	690
Trans-Nerolidol	0.17	704
Isopentyl decanoate	2.25	755
1-Octanol, 2-butyl	0.58	892
Cis-Vaccenic acid	0.12	906
α-Bergamotene	0.55	973
10-Undecyn-1-ol	0.18	990
Propyl palmitate	0.45	998
β-Caryophyllene Epoxide	0.19	1010
Humulane-1,6-dien-3-ol	0.02	1017
Myristic acid, isopropyl ester	0.14	1055
Cis-4,15-Octadecadien-1-ol acetate	0.47	1090
β-Farnesene	0.12	2001
Acoradiene	0.62	2040
Pentadecane	0.18	2094
α-Cedrene	0.41	2039
Germacrene-D-4-ol	0.67	2057
4-Terpineol	7.04	2061
1,8-Cineole	5.62	2078
Sulforous acid, nonyl pentyl ester	0.25	2082
β-Linalol	0.16	2095
9-Decen-1-ol	0.40	2098
Total	92.51	-
Number of compounds	34.00	-
Monoterpenes	38.08	-
Diterpenes	2.17	-
Sesquiterpenes	36.44	-
Triterpenes	1.55	-
Non-terpenes	14.77	-

Table 2. Antioxidant properties of *Duabanga grandiflora* oil

Parameters	Ic50 µg/mL- *Duabanga grandiflora* oil	Ic50 µg/mL (Ascorbic and Gallic acid)
DPPH (% Inhibition)	67.88	21.90
Ferric reducing antioxidant power assay (% Inhibition)	1344.7	810.6
Lipid peroxidation (% Inhibition)	82.15	27.03

Antibacterial activity of *Duabanga grandiflora* oil is presented in Table 3. Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella flexineri* and *Salmonella typhi*) known as causative agents for various diseases in both human and animals. In this study, *Duabanga grandiflora* oil was active against *Bacillus subtilis* (80.00%), *Staphylococcus aureus* (75.00%), *Shigella flexineri* (73.00%), *Escherichia coli* (75.05%), *Salmonella typhi* strain (76.00%) against the reference drug ampicillin with percentage inhibition of 72.00%, 60.00%, 70.00%, 65.00% and 70.00% respectively. However, the oil was no active against *Pseudomonas aeruginosa* strain. Previous report by Mann et al. (2000) has shown that Gram positive bacteria are more susceptible to essential oils than Gram negative bacteria. In this current study, *Duabanga grandiflora* oil showed higher antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Shigella flexineri, Escherichia coli, Salmonella typhi* strains. According to Boussaada et al. (2008), clinical trials have shown that *Pseudomonas aeruginosa* is resistant to antibiotics and less sensitive to essential oils. The antibacterial activity nature of *Duabanga grandiflora* oil could be as a result of the synergy their bioactive compounds which helps to fight inflammation, neutralize free radicals at the source and stimulate the immune system to fight against infections and disease. The result obtained in this study is in agreement with the findings of Bashir et al. (2020).

Table 3. Antibacterial activity of *Duabanga grandiflora* oil

Bacteria	Ampicillin	% Inhibition
Bacillus subtilis	72.00	80.00
Staphylococcus aureus	60.00	75.00
Pseudomonas aeruginosa	65.00	0.00
Salmonella typhi	70.00	76.00
Shigella flexineri	70.00	73.00
Escherichia coli	65.00	75.05

Table 4 reveals the proximate results of *Duabanga grandiflora* leaf. In this study, the moisture of the sample was 13.42 % which is excellent storage. A moisture level of between 8.0-15.0 has been suggested to inhibit the growth of fungi (Sharma *et al*., 2022). *Duabanga grandiflora* leaf contain a crude protein of 15.48 % suggesting that it cannot be used as protein supplement in animals. Crude protein recorded was lower higher than 13.2% for *Terminalia bellerica, Eclipta alba* (11.4%), *Andrographis paniculata* (10.9%), *Bauhinia variegate* (12.6%) and *Terminalia bellerica* (12.2%) reported by Alagbe (2024). Crude fibre and ether extract content of *Duabanga grandiflora* leaf (9.91%) and 0.69% was higher than 7.23% and 0.55% reported by Ali et al. (2020) for *Veronia amygdalina* leaves. The result suggests that *Duabanga grandiflora* leaf could enhance digestion and prevent against cardio-vascular disease (Adewale *et al*., 2021; Alagbe, 2024e). Ash content of a sample can be used to determine their mineral composition. This result indicates that the sample is loaded with abundant minerals necessary for metabolic process (Ojediran *et al*., 2024). The ash composition recorded in this study was lower than 7.08% and 5.62% reported by Sharma et al. (2022) for *Juniperus thurifera* leaf and stem bark respectively. *Duabanga grandiflora* leaf contained 1520.3 Kcal/kg which is comparable to 1500.8 Kcal/kg for *Sida acuta* leaves recorded by Shittu and Alagbe (2020). This result was lower than values recorded for *Pterocapus erinaceus* (1297.5

Kcal/kg) and *Delonix regia* leaves (1300.8 Kcal/kg) reported by Alagbe et al. (2020; 2024).

Table 4. Proximate results of *Duabanga grandiflora* leaf

Parameters	Composition
Moisture	13.42
Crude protein	15.68
Crude fibre	9.91
Ether extract	0.69
Ash	12.07
Energy (Kcal/kg)	1520.3

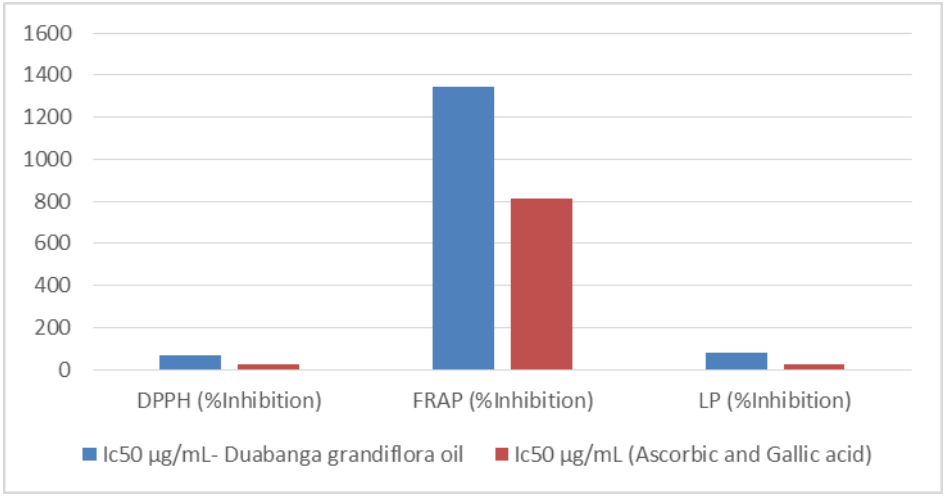


Figure 1. Graphical representation of antioxidant properties of *Duabanga grandiflora* oil

Mineral composition of *Duabanga grandiflora* leaf is presented in Table 5. Potassium had the highest concentration of 909.61 mg/100g followed by calcium (305.24 mg/100g), phosphorus (164.90 mg/100g), sodium (100.3 mg/100g), magnesium (87.32 mg/100g), iron (10.96 mg/100g), zinc (4.51 mg/100g), copper (1.57 mg/100g), nickel (0.25 mg/100g) and cobalt (0.10 mg/100g). The potassium concentration recorded in this study was lower than 607.51 mg/100g reported for *Daniellia oliveri* stem bark by Alagbe et al. (2020). According to Alagbe et al. (2021), *Rauvolfia Vomitoria* leaf contains 406.1 mg/100g of calcium. The variation in the values could be as a result of different locations in which the plants are collected, specie, chemical composition, age of plant as well as method of processing (Agubosi *et al*., 2022; Alagbe, 2024a). Minerals are required for normal growth, activities of muscles and skeletal development (Alagbe *et al*., 2021; Alagbe, 2024b). Copper and Iron are responsible for cellular activity and oxygen transport. Sodium and potassium are needed for chemical reaction in the body and intestinal absorption fluid balance and nerve transmission (Alagbe, 2024c). Calcium and magnesium support bone strength, cardiovascular health and electrolyte balance (Alagbe, 2024d).

Table 5. Mineral composition of *Duabanga grandiflora* leaf

Parameters	Composition (mg/100 g)
Calcium	305.24
Phosphorus	164.90
Potassium	909.61
Magnesium	87.32
Iron	10.96
Zinc	4.51
Manganese	0.68
Copper	1.57
Sodium	100.3
Cobalt	0.10
Nickel	0.25

4. Conclusion

It was concluded that inside *Duabanga grandiflora* are bioactive compounds that go far beyond nutrition. They are natural ally for detoxification, reduce chronic inflammation in animals and neutralize the activities of free radicals. A synergy between these compounds are working together to heal, protect and restore good health in animals. These bioactive compounds are effective and efficient in combating the incidence of antimicrobial resistance.

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