Comparative Phytochemical and Physico-chemical Properties of *Aspilia africana* (Pers) C. D. Adams and *Tithonia diversifolia* (Hemsl) A. Gray Petals as a Scientific Backing to Their Tradomedicinal Potentials

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**Abstract:** The petals of *Aspilia africana* (Pers.) C. D. Adams and *Tithonia diversifolia* (Hemsl.) A. Gray were investigated for their phytochemical constituents, quantitative evaluation, nutritional values, extractive values and wound healing activity. Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, cardiac glycosides in both plant petals. Alkaloids were presence in *T. diversifolia* petals but absent in *A. africana* petals. Anthraquinones was absent in both plant petals. Quantitative evaluation revealed moisture content of 8.5% and 12.3%, total ash of 5.16% and 7.0%, sulfated ash of 4.0% and 5.0%, Acid-insoluble ash of 0.5% and 1.0% for *A. africana* and *T. diversifolia* petals respectively. Nutritional determination revealed 6.5% and 6.0% lipids, 22.0% and 4.0% fibre, 11.8% and 10.06% protein, 59.69% and 79.94% carbohydrate for *A. africana* and *T. diversifolia* petals respectively. Extractive values revealed 15.5% and 16.5% diluted ethanol-soluble, 16.0% and 19.0% water-soluble, 2.5% and 3.5% non-volatile ether-soluble, 2.3% and 2.9% volatile ether-soluble for *A. africana* and *T. diversifolia* petals respectively. Wound healing activity revealed a significantly (p≤0.05) higher rate of wound healing with a percentage wound contraction of 87.50% and 75% for *A. africana* and *T. diversifolia* respectively. The result of these studies indicates that petals extracts possess antioxidant, antibacterial and haemostatic activities which explain its folkloric use in animal nutrition and human medicine.

**Keywords:** *Aspilia africana, Tithonia diversifolia,* petals, phytochemical, Asteraceae.
1. Introduction

Nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plant have long been published but a large number of them remain unexploited as yet. The traditional system of the medicine like Chinese, Ayurvedic, Unani and Biomedicine are very effective particularly in rural areas for the treatment of various ailments. In spite of the advent of modern medicine, tribal populations are still practicing the art of herbal medicine. Nearly about 80% of the total human population still depends upon traditional remedies together with folklore system based mainly on phytotherapy (Azaizeh et al., 2003).

Although the historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public (Matthews et al., 1999). Traditional of healthcare must always been features of human society and from the available evidence it is found that plants are playing a lead role in the therapy (Srivastava, 2000).

A medicinal plant is any plant, which in one or more of its organs contains active ingredients which can be used for the therapeutic purposes or contain foundation compounds that can be used for the synthesis of useful drugs (Sofowora, 2008).

It has also been reported that, the African continent have a long history with the use of plants and in some African countries, up to 90% of the population rely on medicinal plants as a source of drugs (Hostettmann et al., 2000).

At a time when majority of mankind has moved away from life’s natural ways, when illness, caused by a changed attitude to life threatens, we should turn again to medicinal herbs which God in His greatness have provided for us since time immemorial (Odoemena and Egwali, 2006). Thus, plants such as *Aspilia africana* and *Tithonia diversifolia* in the family Asteraceae used in herbal medicines to cure diseases and heal injuries is due to its tradomedicinal potentials.

The crushed leaves and flowers of *A. africana* is widely used in ethnomedicinal practice in Africa for its ability to stop bleeding, as well as promote rapid healing of wounds and sore and for the management of problems to cardiovascular diseases (Dimo et al., 2002). The leaves and flowers of *A. africana* are also used in the treatment of rheumatic pain (Oliver, 1960) as well as bee and scorpion stings (Single, 1965).

In Kenya, they are used to kill intestinal worms. In Uganda, it is used to treat gonorrhea (Page et al., 1992). In some community in Nigeria, women boil and filter the leaves of *A. africana* which they drink to prevent conception (Eweka, 2008). The plant has a wide reputation and use as a haemostatic, and in Liberia it is even credited with the capacity of arresting bleeding of severed artery (Dalziel, 1955). The leaf-sap is also an eye – medicine in Tanganyika, dripped into the eyes for eye-
pains of no apparent origin. The leaf-sap and a leaf-decoction are rubbed onto the breasts and made in an inhalation in Tanganyika to promote milk-flow. The fresh crushed leaves and flowers are used. Their application is said to draw up exudations and to promote rapid healing. Amongst the Hausa, superstitious uses are prominent as a love-philtre, and a charm prepared from the plant and tied around the forehead attracts the “glad eye” (Burkill, 1985).

The stems and leaves of *T. diversifolia* are used as a fodder (Roothaert and Paterson, 1997), Poultry feed (Odunsi et al., 1996). In Nigeria, the decoctions of various parts are used for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains (Elufioye and Agbedahunsi, 2004; Owoyele et al., 2004; Moronkola et al., 2007).

Oral decoction of the leaves and stem is used for the treatment of hepatitis in Taiwan and gastrointestinal disorder in Kenya and Thailand (Johns et al., 1995).

*Tithonia diversifolia* (Hemsl) A. Gray, green biomass have been recognized as an effective source of nutrients for lowland rice (*Oryza sativa*) in Asia and more recently for maize (*Zea mays*) and vegetations in eastern and southern Africa (Sao et al., 2010).

Mahecha and Rosales (2005) stated that, the protein in *T. diversifolia* foliage is highly soluble and hence is quickly fermented in the rumen of goats.

Liasu and Ayandele (2008) reported on the antibacterial activity of aqueous extract of *T. diversifolia* stem and ethanolic extract of its flower. It has been reported that *A. africana* has some contraceptive or anti-fertility properties (Eweka, 2007).

Olowokudejo et al. (2008) recorded an ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria whereby Aspilia africana leaves and flowers were used as haemostatic, cleaning sores, corneal opacities, stomach disorders, tuberculosis, neuron’s disorders, guineacrosom, gonorrhoea and skin rashes.

From available literature, several work have been carried out on *A. africana* and *T. diversifolia*, but there has yet being no report on the “petals” of the flower of these two plants as a scientific backing to its tradomedicinal potentials.

The present study is to carry out a comparative phytochemical screening to determine the different bioactive agents in the “petals” of *A. africana* and *T. diversifolia* flower; determine the proximate composition of the “petals” of the two plants and determine the amount of constituents which is extractable using different solvent (i.e. extractives) under the specified condition.

The significance of this study is to prove that the therapeutic uses of the petals of these *A. africana* and *T. diversifolia* flowers will be considerably clarified from these pieces of information.

2. Materials and Methods
The fresh flower (petals) of *Aspilia africana* (Pers) C. D. Adams and *Tithonia diversifolia* (Hensl) A. Gray were collected from a bush in Okobo, Oron, and Uyo Local government Area of Akwa Ibom State. The plant was authenticated by Dr. (Mrs.) U. A. Essiett, a taxonomist in the department of Botany and Ecological Studies, University of Uyo, Uyo.

2.1. Preparation of the Extract

After collection and identification of the two plant flowers, the petals were then separated from the flower, sun dried and then powdered. The powdered material were weighed accurately and 20g each were macerated cold in 400mL of 70% ethanol for 72 hours (3 days) in a maceration tank at room temperature following Sofowora (2008) method. The plant materials were then filtered and the filtrate obtained was concentrated in water bath at 40ºC which yield a semi-solid extract that is then used for the phytochemical screening. 20g of 400mL ethanolic extraction of *A. africana* petals and *T. diversifolia* petals yields 18.4% and 18.9% extract respectively. The extract was used to carry out phytochemical screening using a suitable method (Trease and Evans, 1989; Sofowora, 2008).

2.2. Quantitative Microscopy/Proximate Analysis

The moisture content of the powdered leaves was determined by loss on drying method (African Pharmacopoeia, 1986). The ash value, acid insoluble ash, water-soluble ash and sulphated ash were determined as described (British Pharmacopoeia, 1980; African Pharmacopoeia, 1986). The water and alcohol extractive values were obtained using the method outlined (Brain and Turner, 1975; British Pharmacopoeia, 1980). The fat (lipids), crude fibre, crude protein and carbohydrate were obtained using the method outlined (Pearson, 1976; Okon, 2005; AOAC, 2000).

3. Results and Discussion

3.1. Phytochemical Screening

The result of preliminary phytochemical analysis of the petals of *Aspilia africana* and *Tithonia diversifolia* flower shows the presence of saponins, tannins, flavonoids and cardiac glycosides. Alkaloids was found to be absent in petals of *A. africana* flower, but present in petals of *T. diversifolia* flower. Anthraquinones were found to be absent in the phytochemical screening of the petals of the plant (Table 1).

3.2. Proximate Analysis

The result of the proximate analysis (quantitative evaluation in Table 2 and nutritional analysis in Table 3) of the petals of *A. africana* and *T. diversifolia* flower show moisture content (8.5% and
12.3%), Ash content (5.16% and 7.0%), Sulfated ash (4.0% and 5.0%), Acid – insoluble ash (0.5% and 1.0%) respectively as quantitative evaluation (Table 2). Lipid (6.5% and 6.0%) Fibre (22.0% and 4.0%), Protein (11.8% and 10.06%) and carbohydrate (59.69% and 79.94) as nutrients values for A. africana and T. diversifolia petals respectively (Table 3).

**Table 1: Result of the phytochemical analysis of the ethanolic extract of A. africana and T. diversifolia petals**

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Inferences</th>
<th>A. africana</th>
<th>T. diversifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins (frothing test)</td>
<td>Frothing persisted for more than 10mins.</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>A blue-black colouration was observed</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>A cleared solution was observed in A. africana and orange ppt colouration was observed in T. diversifolia</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Orange colouration was observed</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Lieberman’s test</td>
<td>A blue-green colour was formed</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Salkowski’s test</td>
<td>A reddish-brown colour was observed at the interface</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Keller-killiani test</td>
<td>A brown ring was observed at the interphase</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones test</td>
<td>No purple-pink colouration was observed at the mixable layer</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Key**
- Absent = -
- Trace = +
- Moderately = ++
- Abundantly present = +++

### 3.3. Extractives

The result of extractives also shows percentage extractive values of A. africana and T. diversifolia flower petals. Diluted Alcohol – soluble (15.5% and 16.5%), water – soluble (16.0% and 19.0%), Non – volatile Ether – soluble (2.5% and 3.5%) and volatile ether – soluble (2.3% and 2.9%) respectively (Table 4).
Table 2: Result of Quantitative Evaluation of the Petals of *A. africana* and *T. diversifolia*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage Composition (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. africana</em></td>
</tr>
<tr>
<td>Moisture content</td>
<td>8.5</td>
</tr>
<tr>
<td>Ash content</td>
<td>5.16</td>
</tr>
<tr>
<td>Sulfated ash</td>
<td>4.0</td>
</tr>
<tr>
<td>Acid – insoluble ash</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3: Result of the Nutritional Analysis of the Petals of *A. africana* and *T. diversifolia*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage Composition (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. africana</em></td>
</tr>
<tr>
<td>Lipid</td>
<td>6.5</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>22</td>
</tr>
<tr>
<td>Crude protein</td>
<td>11.81</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>59.69</td>
</tr>
</tbody>
</table>

Table 4: Result of the extractives of the petals of *A. africana* and *T. diversifolia*

<table>
<thead>
<tr>
<th>Extractives</th>
<th>Values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. africana</em></td>
</tr>
<tr>
<td>Diluted ethanol-soluble</td>
<td>15.5</td>
</tr>
<tr>
<td>Water – soluble</td>
<td>16.0</td>
</tr>
<tr>
<td>Non-volatile ether-soluble</td>
<td>2.5</td>
</tr>
<tr>
<td>Volatile Ether-soluble</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The result of phytochemical analysis revealed the presence of alkaloids in trace quantity in *T. diversifolia* petals which primarily acts as feeding deterants and toxin to insects and other herbivores (Harborne, 1993), in many cases by directly interacting with molecular targets within the nervous system (Wink, 2000), and are toxic to mammals (Rattan, 2010). This property was absent in *A. africana* petals.

Flavonoids exhibit a broad range of biological activity such as antimicrobial, anticancer, antiallergic and well as antitumour properties. On the other hand, flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Pietta, 2000). Flavonoids in intestinal tract lower the risk of heart disease, and as antioxidant,
it provide anti-inflammatory activity (Okwu, 2004; Essiett et al., 2010). This may be the reason why A. africana and T. diversifolia petals have been used for the treatment of wounds, burns and ulcer in herbal medicine. Flavonoids in both plants petals is an important component of a normal diet, and has been suggested that flavoid-rich foods may limit neurodegeneration and prevent or reverse normal or abnormal deterioration in cognitive performance (Spencer, 2010).

Saponins which were present in abundant quantity in A. africana and moderate quantity in T. diversifolia petals have anti-inflammatory, anti-yeast, anti-fungal, anti-tumours, anti-parasitic and antiviral activities that confirms its usefulness in tradomedical practitioners (Sofowora, 2008; Sparg et al., 2004). It has the property of precipitating and coagulating red blood cells and some characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). These properties bestow high medicinal activities on the extracts from A. africana and T. diversifolia petals.

Tannins which have astringent and detergent properties were also present in trace quantity in both plants petals, and this could be responsible for their anti-fungal activity when used against diarrhoea and malaria. This coincides with reported works (Bouquet et al., 2008; Trease and Evans, 2002; Bruneton, 1999).

There has been an assertion by Trease and Evans (2002) that naturally cardiac glycosides are used for treatment of various diseases associated with the heart such as in controlling supraventricular (atrial) cardiac arrhythmias, it also exert a slowing and strengthening effect on failing heart. Cardiac glycosides were also found to be present in both petals of A. africana and T. diversifolia flower extracts while anthraquinones were absent in both plants petals. The presence of this compound in A. africana and T. diversifolia petals could be useful in the treatment of diseases associated with the heart (Trease and Evans, 2002).

The presence of these phytochemicals explains why petals of A. africana and T. diversifolia are used by tradomedical practitioners to stop bleeding, cure wounds, allergies, rheumatism, inflammatory and ulcers.

The proximate analysis, quantitative evaluation (Table 2) and nutritional values (Table 3), is an important parameter in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterants in the drugs. The moisture content that is not too high indicates less chances of microbial degradation of the drugs during storage and in this study, the moisture content value for A. africana petals was 8.5% and that of T. diversifolia petals was 12.3% and the general requirement for moisture content in a crude drug is not more than 14% (British Pharmacopoeia, 1980), thus, the values obtained in this research work is within the accepted range and this is an agreement with Edward et al., 1971; Musa et al., 2005; Schuna, 2010.
The total ash which is the measurement of the amount of the residual substances not volatilized when the drug sample is ignited by heat. Ash may be derived from the plant tissue itself (i.e. physiological ash) or from the extraneous matter, especially sand and soil adhering to the surface of the drugs (i.e. non-physiological ash), and this kinds of ash are determined together, therefore it is referred to as total ash (African Pharmacopoeia, 1986). A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing (Chandel et al., 2011). In this work, the total ash values of 5.16% and 7.0% for Aspilia africana and Tithonia diversifolia petals respectively were found to be reasonably low, indicating low contamination when stored.

The determination of acid-insoluble ash is a method intended to measure the amount of silica especially sand and silicious earth present in the drugs and the percentage of silica was found to be 0.5% for A. africana and 1.0% for T. diversifolia petals which implies that a small portion of the ash content is acid soluble, it also indicates that T. diversifolia petals is more digestible than A. africana petals since a high value indicate a high digestibility of the plant when eaten or consumed (Okhale et al., 2010; Ibrahim et al., 2010). The sulfated ash of 4.0% for A. africana and 5.0% for T. diversifolia petals are good criteria to judge the identity and purity of crude drug.

Protein which is the major compound containing nitrogen in any food sample is used as an index of protein termed “crude protein” as distinct from true protein (Okon, 2005). In this research work, protein content of 11.81% for A. africana and 10.06% for T. diversifolia petals shows that A. africana petals are riched in protein than T. diversifolia petals when consumed.

Fat (Lipid) content of 6.5% for A. africana petals is higher than (6.0%) that of T. diversifolia petals, and this indicates that A. africana petals have high source of energy than T. diversifolia petals and this high energy are needed especially by growing animals. By definition, fat is the materials soluble in certain organic solvent, but the presence of bound fat such as Lipoprotein, create problems (Okon, 2005).

Fibre content of 22% for A. africana and 4% for T. diversifolia petals indicate that A. africana petals have more excessive woody materials than T. diversifolia petals, hence, fibre determination is the criteria for judging purity of herbal crude drugs (Agarwal, 2005), and carbohydrate content of 59.69% for A. africana petals is lower than that of T. diversifolia petals which is 79.94% (Table 3).

Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicate addition of exhausted material, adulteration or incorrect processing during drying or storage (Chandel et al., 2011). The water-soluble extractive values for A. africana and T. diversifolia petals were 16% and 19% respectively (Table 4). Diluted alcohol-soluble extractive values
of 15.5% for \textit{A. africana} petals and 19% for \textit{T. diversifolia} petals was also indicative for the same purposes as the water-soluble extractive.

The ether-soluble extractive value signifies the presence of amounts of fats, lipids and some steroids in the drug (Chandel \textit{et al.}, 2011). In this research work, ether-soluble extractive values for \textit{A. africana} and \textit{T. diversifolia} petals were in the range of 2.3% - 3.5% (Table 4). \textit{A. africana} petals have 2.5% non-volatile ether-soluble extractive value and 2.3% volatile ether-soluble extractive value, while \textit{T. diversifolia} petals have 3.5% non-volatile ether-soluble extractive value and 2.9% volatile ether-soluble extractive value. Comparing the water-soluble, dilute alcohol-soluble and ether-soluble (non-volatile and volatile) extractive values of the drugs (i.e. \textit{A. africana} and \textit{T. diversifolia} petals), it was observed that the percentage water-soluble extractive values were higher than the percentage dilute alcohol-soluble and ether-soluble extractive; this indicates presence of more amounts of water-soluble contents in the two plants petals. This means that water will be a better solvent for extraction of the plant.

4. Conclusion

In conclusion, the traditional knowledge on \textit{A. africana} and \textit{T. diversifolia} shows that the plants are reputable for the treatment of heart diseases, wound healing, malaria, prevent conception and also in the production of antimalaria, antidiabetes, anti-inflammatory and anti-fungal drugs due to its chemical constituents.

The analysis carried out on these plants shows that \textit{A. africana} and \textit{T. diversifolia} petals are rich in secondary metabolites which could be explored as potentials drugs and phytomedicines. Thus, the research and its result could serve as a scientific backing to the use of \textit{A. africana} (Pers) C. D. Adams and \textit{T. diversifolia} (Hemsl) A. Gray petals in tradomedicinal practices for cure of different ailments.

5. Recommendation

Having reached the conclusive part of this work, it is therefore recommended that \textit{A. africana} (Pers) C. D. Adams and \textit{T. diversifolia} (Hemsl) A. Gray should be used as medicine for its efficacy for curing different ailments and further studies should be carried out on the petals of \textit{A. africana} and \textit{T. diversifolia} to determine the extent and dosage at which a patient should use. Studies should also be carried out on antinutrient, mineral element, saponification value, iodine value, chemo-microscopical examination and thin-layer chromatography for the development of new drugs.
References


