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Comparative Phytochemical Composition and *In vitro* Antioxidant Capacity of Aqueous and Methanolic *Gmelina arborea* Leaf Extracts

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Abstract

This study evaluated and compared the phytochemical composition and in vitro antioxidant potential of aqueous Gmelina arborea *leaf extracts (AGALE) and methanolic* Gmelina arborea *leaf extracts (MGALE). Qualitative and quantitative phytochemical analysis was carried out in both extracts. Both extracts were found to contain key bioactive compounds such as tannins, phenols, flavonoids, alkaloids, and saponins. However, notable differences were observed in their concentration levels: MGALE exhibited higher ($P<0.05$) contents of phenols (110.0 ± 0.1 mg/100 mL), flavonoids (1378 ± 13.0 mg/100 mL), and alkaloids (425.0 ± 0.2 mg/100 mL), whereas AGALE showed greater levels of tannins (103.88 ± 0.12 mg/100 mL) and saponins (1010 ± 20 mg/100 mL). The antioxidant activities of both extracts were evaluated using 1, 1-diphenyl-2-picryl-hydrazyl assay; (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); (ABTS) and the ferric reducing antioxidant power (FRAP) assay. Results showed that MGALE exhibited significantly higher ($P<0.05$) antioxidant activity, with DPPH (54.89 ± 0.08%) and ABTS (75.93 ± 0.25%) values, compared to AGALE, which recorded 33.92 ± 0.16% and 27.48 ± 0.11%, respectively. Conversely, AGALE exhibited a markedly ($P<0.05$) superior FRAP: 50.00 ± 0.11% compared to MGALE (12.65 ± 0.03%). The study concludes that both AGALE and MGALE have valuable potential in ruminant nutrition and health, and that the extraction solvent is a key factor influencing the concentration and effectiveness of their bioactive components.*

***Keywords**: Secondary metabolites, Bioactive, Ethno-medicine, Antioxidant activity, Methanolic extraction, Aqueous extraction*

Introduction

Plants remain a vital source of therapeutic agents and continue to serve as the foundation of traditional and modern medicine due to their affordability, accessibility, and minimal side effects. In many developing countries, increasing attention has been directed toward the documentation of traditional medicinal knowledge and the scientific evaluation of medicinal plants for the development of herbal remedies (Wight, 2018). Virtually all plants contain bioactive compounds with antimicrobial or pharmacological potential (Khan *et al.,* 2011). These compounds form an important basis for drug discovery and the development of new therapeutic agents for the management of various diseases (Adegbesan, 2019).

Phytochemical constituents of plants are not only important for the identification of novel drug candidates but also serve as sources of economically valuable natural compounds for chemical synthesis and industrial applications. The diverse phytochemicals present in plants often exhibit complementary or synergistic effects that enhance their biological activity. Consequently, the scientific validation and standardization of herbal medicines have become essential areas of research, aimed at ensuring the safety, efficacy,

and reproducibility of natural products (Nida and Rahimullah, 2022). Plant-derived compounds have long been utilized in the treatment of a wide range of diseases, including infectious and metabolic disorders (Roy *et al.,* 2024).

Gmelina arborea Roxb. (family Verbenaceae), commonly known as beechwood or white teak, is a fast-growing deciduous tree native to tropical and subtropical Asia. It has been widely introduced in West Africa, particularly in Nigeria and Côte d'Ivoire, where it is valued for both its medicinal and fodder potential (Tiwari *et al.,* 2008). Ethnobotanical reports indicate that *G. arborea* possesses multiple medicinal properties, including aphrodisiac, astringent, diuretic, analgesic, and tonic effects (Doughari *et al.,* 2007). The leaves have been traditionally used in the treatment of hypertension, malaria, and insect or scorpion stings, while the stem bark and fruits are applied in managing diarrhea, wounds, and fever (Moronkola *et al.,* 2001; Nayak *et al.,* 2011).

Phytochemical screening of *G. arborea* has revealed the presence of several secondary metabolites such as alkaloids, flavonoids, saponins, glycosides, tannins, phenolic compounds, steroids, and triterpenoids, which are associated with antimicrobial, antioxidant, and anti-inflammatory properties (N'gaman *et al.,* 2009; Nayak *et al.,* 2012). The plant has demonstrated significant antimicrobial activity, validating its ethno-medicinal use as a broad-spectrum therapeutic agent (Prashanth *et al.,* 2006). Additionally, its fruits and leaves have shown antibacterial potential, supporting their use in the management of wounds, sores, burns, and other inflammatory conditions (Akyala *et al.,* 2013).

Phytochemicals are biologically active secondary metabolites that interact with dietary nutrients and fibres to promote health and prevent disease (Sangeeta *et al.,* 2006). These compounds are responsible for the colour, flavour, and aroma of plants and have been associated with the prevention of degenerative and chronic diseases. Based on their chemical structures, phytochemicals are broadly classified into phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids are further divided into subgroups such as anthocyanins, flavones, flavanones, isoflavones, and flavonols (Arts and Hollman, 2005).

Although *Gmelina arborea* is widely recognized for its medicinal value and is traditionally used for various therapeutic purposes, there is limited scientific evidence comparing how different extraction solvents influence its phytochemical and antioxidant properties. This lack of comparative data creates a gap in understanding the optimal extraction method for maximizing its bioactive potential.

Therefore, the objective of this study is to compare the phytochemical composition and antioxidant activities of *G. arborea* leaf extracts obtained using water and methanol, in order to identify solvent-related differences and provide scientific support for its traditional and potential pharmaceutical applications.

Materials and Methods

Sourcing of *Gmelina arborea* Leaf

A total of 50 kg of fresh *Gmelina arborea* leaves was collected from the Botanical Garden of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Only healthy, disease-free leaves were selected. The leaves were thoroughly washed to remove dirt and debris, then air-dried under shade to prevent loss of phytochemicals from direct sunlight. The dried leaves were subsequently ground into a fine powder to increase surface area and improve extraction efficiency.

Preparation of Aqueous and Methanolic *Gmelina arborea* Leaf Extract

Aqueous extract was prepared by soaking a total of 30 kg of *Gmelina arborea* leaf meal in 45 L of distilled water for 24 hours. The mixture was first strained through a muslin cloth and then filtered using Whatman No. 1 filter paper. The filtrate was concentrated

using a rotary evaporator equipped with a water bath, condenser, sender flask, receiver flask, pressure system, and control unit. The filtrate was placed in the sender flask, and the water bath temperature was maintained at 50 °C to prevent heat degradation. As the machine operated, vapor from the filtrate condensed and collected in the receiver flask. Once the extract thickened, it was transferred into Petri dishes. The semi-solid extract was then freeze-dried to obtain a crystalline aqueous *Gmelina arborea* leaf extract.

The methanolic extract was prepared by placing 20 kg of *Gmelina arborea* leaf meal in a glass container and adding 75 L of 70% methanol. The mixture was stirred at two-hour intervals and allowed to extract for 72 hours. The solvent mixture was then strained through a muslin cloth and further filtered using Whatman No. 1 filter paper. The remaining residue was re-extracted with an additional 75 L of 70% methanol using the same procedure. The combined filtrates were concentrated using a rotary evaporator (Heidolph Laboratory 400 Efficient, Model 517-01002-002) set at 40 °C, after which the concentrate was dried in a vacuum oven at 40 °C and 700 mmHg to obtain the methanolic extract of *Gmelina arborea* leaves.

Qualitative and Quantitative Phytochemical Screening

Aqueous and Methanolic extract of *Gmelina arborea* leaf meal were subjected to preliminary screening of phytochemical constituents. The procedures were described by Idowu *et al.* (2024).

Determination of tannin Content

0.1g of the sample was added 10mL of distilled water in a test tube and filtered. 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

The tannins were determined by Folin - Ciocalteu method. The sample was prepared and about 0.1 mL was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteuphenol reagent, 1 mL of 35% Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract

Determination of saponins

0.1g of the sample was added 10mL of distilled water and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion which indicates the presence of saponins.

2.0g of the sample was weighed into a 250mL conical flask. 100 mL of 20% C_2H_5OH was added. The mixture was then heated over a hot water bath for 4 hours with continuous stirring at about 55°C and filtered with a filter paper. The residue was re-extracted with another 200mL of 20% C_2H_5OH. The combined extract was then reduced to 40ml over a water bath at about 90°C. The concentrated extract was transferred into a 250mL separator funnel and 20mL of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the $(CH_3CH_2)_2O$ layer discarded. This purification process was repeated. 60ml of n-butanol added and the combined n-butanol extract was washed twice with 10mL of 5% NaCl solution. The remaining solution was then heated on a water-bath in a pre-weighed 250mL beaker. After evaporation, the residue was dried in an oven to a constant weight. The % saponins was then calculated by difference.

Determination of flavonoids

A few drops of 1% NH_3 solution was added to the aqueous and methanolic extract of the sample in a test tube. A yellow coloration which disappears with the addition of

concentrated sulphuric acid was observed if flavonoid compounds are present.

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 mL of sample prepared and 4 mL of distilled water taken in a 10 mL volumetric flask. To the flask, 0.30 mL of 5 % sodium nitrite was treated and after 5 minutes, 0.3 mL of 10 % aluminium chloride was mixed. After 5 minutes, 2 mL of 1M Sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Determination of alkaloids

0.1 g of the sample was diluted to 10 mL with acid alcohol and filtered. To 5 mL of the filtrate was added 2 mL of dilute ammonia. 5mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

2g of the sample was placed in a 250mL beaker and 200mL of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate concentrated on a water bath until it reached a quarter of its original volume. Concentrated NH_4OH was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate alkaloid was dried and weighed.

Determination of total phenol content

0.1g of the sample was dissolved in a test tube with ethanol, shook and filtered into another test tube and few drops of ferric chloride added and observed for a green or blue-black colouration which indicates the presence of phenol

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Lee *et al.,* 2008). The sample was prepared and 0.5 ml of different dilutions were mixed with Folin Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 mL, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared and solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values were expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound.

Determination of Antioxidant Activity: DPPH Free Radical

Antioxidant screening of the leaf extract was done according to the procedure of Lee *et al.* (2003) The antioxidant properties of *Gmelina arborea* leaf extracts were assessed using the DPPH free radical scavenging assay as described by Manzocco *et al.* (1998). The extracts were serially diluted to concentrations of 10, 20, 40, 60, 80 and 100 µg/mL. For each concentration, 1 mL of the extract was mixed with 1 mL of 0.004% DPPH solution (prepared in ethanol) and incubated at 37°C for 30 minutes. The absorbance of the reaction mixture was then measured at 517 nm. Absolute methanol served as the negative control.

DPPH radical scavenging activity (%) was calculated using the formula:

$$\text{DPPH Scavenging Activity (\%)} = \frac{Ao-A}{A0} \times 100$$

Where

A = absorbance of sample with extract

A_0 = absorbance of negative control (0.004% DPPH solution)

Ascorbic acid was used as the positive control. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was

determined by plotting percentage scavenging activity against extract concentration. Determination of Ferric Reducing Antioxidant Potential (FRAP) was according to the method of Benzie and Strain, (1996).

AABTS Free Radical Scavenging Method

The antioxidant activity of aqueous and methanolic extract of *Gmelina arborea* leaf meal against ABTS was determined by the method described by Strati *et al.* (2006). Radical $ABTS^{\bullet+}$ was prepared through oxidation of ABTS by potassium persulfate. A mixture (1:1; *v*/*v*) of ABTS (7 mM) and potassium persulfate (4.95 mM) was prepared and kept in the dark for 16 h at room temperature. Then, the mixture was diluted with methanol until it reached absorbance values of 1–1.5 at 734 nm. Aliquots of 0.1 mL of methanolic extract of each sample (at 4 different concentrations: 0.1, 0.5, 1, and 2 mg/mL; two replicates per sample and concentration) had 3.9 mL of the $ABTS^{\bullet+}$ dilution added. The absorbance decrease was measured at 734 nm in a UV-30 spectrophotometer. The blank was prepared with $ABTS^{\bullet+}$.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to the method of Benzie and Strain (1996). A 0.2 mL aliquot of each aqueous and methanolic extract prepared at concentrations of 0.1, 0.5, 1.0, and 2.0 mg/mL in duplicate was mixed with 3.8 mL of freshly prepared FRAP reagent. The reagent consisted of 10 parts of 300 mM sodium acetate buffer (pH 3.6), 1 part of 10 mM TPTZ solution, and 1 part of 20 mM $FeCl_3 \cdot 6H_2O$ (Alfa Aesar, Kandel, Germany). The reaction mixture was incubated at 37 °C for 30 minutes, after which absorbance was recorded at 593 nm using a UV-30 spectrophotometer (GIORGIO-BORMAC SRL, Carpi, Italy). A blank was prepared by replacing the extract with methanol. Results were expressed as milligram equivalents of $FeSO_4$ per milligram of dry weight. Calibration was performed using $FeSO_4$ standards at concentrations of 0.0025, 0.005, 0.01, and 0.02 mg/mL.

Data Analysis:

Data collected in triplicate were pooled and expressed as mean ± standard deviation, then analyzed using SPSS (Statistical Package for Social Science) software version 2017. Student t-test was carried out to ascertain the level of significance at 0.05.

Results

The comparative quantitative phytochemical analysis of aqueous and methanolic *Gmelina arborea* leaf extract is shown on Table 1. It was observed that tannins, phenols, flavonoids, alkaloids, and saponins were present in *Gmelina arborea* leaf extract. The quantitative phytochemical screening of *Gmelina arborea* leaves revealed marked variations between methanolic and aqueous extracts. In the methanolic extract, flavonoids were found in the highest concentration (1378.0 ± 13.0 mg/100 mL), followed by saponins (640.0 ± 0.4 mg/100 mL), alkaloids (425.0 ± 0.2 mg/100 mL), phenols (110.0 ± 0.1 mg/100 mL), and tannins (96.0 ± 0.3 mg/100 mL). In contrast, the aqueous extract contained higher levels of saponins (1010.0 ± 20 mg/100 mL) and tannins (103.88 ± 0.12 mg/100 mL), but relatively lower flavonoids (117.01 ± 0.33 mg/100 mL) and phenols (76.25 ± 0.35 mg/100 mL). Alkaloid content in the aqueous extract was recorded at (330.0 ± 10 mg/100 mL).

Table 1: Quantitative phytochemical analysis of aqueous and methanolic *Gmelina arborea* leaf extract

Parameters (mg/100mL)	AGALE	MGALE
Tannin	103.88 ± 0.12[a]	96.0 ± 0.3[b]
Phenols	76.25 ± 0.35[b]	110.0 ± 0.1[a]
Flavonoids	117.01 ± 0.33[b]	1378 ± 13.0[a]
Alkaloids	330 ± 10[b]	425.0 ± 0.2[a]
Saponins	1010 ± 20[a]	640.0 ± 0.4[b]

[a,b] *means along the same row with different superscripts are significant different*, AGALE: Aqueous *Gmelina arborea Leaf extract* Quantitative Extract, MGALE: Methanolic *Gmelina arborea Leaf extract*

Table 2 shows the comparative qualitative phytochemical analysis of aqueous and methanolic *Gmelina arborea* leaf extract. The qualitative analysis confirmed the presence of all tested phytochemicals in both extracts, but at varying intensities. Methanol extract showed moderate presence (++) for tannins, phenols, flavonoids, alkaloids, and saponins, while aqueous extract showed weaker detection (+) for tannins, phenols, alkaloids, and saponins, but a moderate presence (++) for flavonoids.

Table 2: Qualitative phytochemical analysis of aqueous and methanolic *Gmelina arborea* leaf extract

Parameters	AGALE	MGALE
Tannin	+	++
Phenols	+	++
Flavonoids	++	++
Alkaloids	+	++
Saponin	+	++

Present +, Moderately present: ++

Indicated in Table 3 is the comparative antioxidant activities of aqueous and methanolic *Gmelina arborea* leaf. The antioxidant evaluation of *Gmelina arborea* leaf extracts revealed significant variations between the methanolic and aqueous extracts. The methanolic extract exhibited higher DPPH scavenging activity (54.89 ± 0.08%) compared to the aqueous extract (33.92 ± 0.16%), indicating a stronger capacity to neutralize free radicals. Similarly, the ABTS assay showed superior activity in the methanolic extract (75.93 ± 0.25%) relative to the aqueous extract (27.48 ± 0.11%). These results suggest that methanol effectively extracted a higher concentration of antioxidant phytochemicals such as flavonoids and phenolic compounds, which are known contributors to radical scavenging. Conversely, the aqueous extract demonstrated the highest FRAP value (50.00 ± 0.11%) compared to the methanolic extract (12.65 ± 0.03%), indicating its greater ferric reducing antioxidant power. This suggests that while methanol extracts were more efficient in scavenging free radicals, the aqueous extract had stronger reducing capacity, possibly due to the solubility of certain hydrophilic antioxidants in water.

Table 3: Comparative Antioxidant activities of Aqueous and Methanolic *Gmelina arborea* leaf

Parameters	Aqueous Extract (%)	Methanolic Extract (%)
Total antioxidant (DPPH scavenging)	33.92 ± 0.16	54.89 ± 0.08
ABTS	27.47 ± 0.11	75.93 ± 0.25
FRAP (%)	50.00 ± 0.11	12.65 ± 0.03

DPPH (1, 1'-diphenyl-2-picryl-hydrazyl assay), ABTS: 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) FRAP (Ferric reducing antioxidant power)

Discussion

The present study compared the phytochemical profiles of aqueous and methanolic extracts of *Gmelina arborea* leaves, and further evaluated the antioxidant activity of the aqueous and methanolic extract. The findings demonstrated that methanol was more efficient than water in extracting major secondary metabolites such as flavonoids, tannins, phenolics, and alkaloids. This was in line with the results reported by Edeoga *et al*. (2005), who highlighted higher yields of alkaloids and polyphenols with methanol compared to aqueous extraction. This result agrees with the observations of previous researchers (Olubodun and Osagie, (2016); Akinmoladun *et al*. (2020); Olubodun *et al.* (2021)) who reported the presence of some phytochemicals in different solvent extracts of the leaves, fruit or other plant parts of *A. boonei* and other plant species. The variations in the plant materials, the range of chemical components in the plant materials and their varying solubility characteristics in various solvents, the chemicals to be extracted, and/or extraction techniques could all contribute to the observed variations (Olubodun *et al.,* 2021). The study revealed that methanol is better for extraction of phytochemicals in *Gmelina arborea* leaf meal when compared with aqueous extraction.

Phytochemicals exhibit varied biochemical and pharmacological properties and have been shown to ameliorate diverse health issues because of its antioxidant activity (Ali *et al.,* 2019). The possible antioxidant activities of phytochemicals were proposed to be the reason for their medicinal effects since oxidative stress is implicated in most disease conditions (Nkono *et al.,* 2014). The antioxidant activities of *Gmelina arborea* leaf extracts varied significantly between the solvents. The methanolic extract showed higher ($P<0.05$) DPPH scavenging activity ($54.89 \pm 0.08\%$) and ABTS inhibition ($75.93 \pm 0.25\%$) compared to the aqueous extract ($33.92 \pm 0.16\%$ and $27.48 \pm 0.11\%$, respectively). The results showed that both extracts demonstrated DPPH radical scavenging activity, with the methanolic extract exhibiting significantly higher ($p < 0.05$) activity than the aqueous extract across all concentrations. As the concentration of the methanol extract increased, its percentage DPPH scavenging activity also increased. This was accompanied by a corresponding decrease in DPPH absorbance, indicating effective neutralization of free radicals. These findings suggest that the extracts contain phytochemicals capable of reducing DPPH and scavenging free radicals (Olubodun *et al.,* 2021).

Conversely, the aqueous extract exhibited stronger ferric reducing antioxidant power (FRAP) ($100 \pm 0.11\%$) compared to the methanolic extract ($12.65 \pm 0.03\%$), suggesting that hydrophilic antioxidants were more concentrated in water (Sulaiman *et al.,* 2015). Ferric Reducing Antioxidant Potential (FRAP) is directly proportional to the molar concentration of antioxidants present in biological samples. The results showed significant differences between the extracts, with the aqueous extract displaying a higher ($P< 0.05$) FRAP value than the methanolic extract. Antioxidant activity reflects a compound's reducing ability, as antioxidants convert Fe^{3+} to its ferric form (Rahman *et al.,* 2015). The methanol extract showed a slight increase in reducing power with concentration, while the aqueous extract remained stable. These findings align with previous reports on the antioxidant effects of *A. boonei* (Omoregie *et al.,* 2014; Obiagwu *et al.,* 2014), suggesting that its antioxidant activity may underlie its medicinal properties.

In the context of animal production, these antioxidant properties are highly beneficial. Antioxidants reduce oxidative stress in livestock, improving growth performance, reproductive efficiency, and disease resistance (Surai, 2016). Plant-derived antioxidants such as those in *Gmelina arborea* can enhance immune function, lower incidences of metabolic disorders, and improve meat and milk quality by preventing lipid peroxidation (Balogun *et al.,* 2019). The complementary activity of methanolic and aqueous extracts implies that both extracts could serve as natural

feed additives, reducing reliance on synthetic antioxidants and antibiotics while promoting sustainable livestock production.

Conclusion

It can therefore be concluded from the findings of this study that:

- The aqueous *Gmelina arborea* leaf extract contained generally lower levels of phytochemicals than the methanolic extract, except for tannins and saponins, which were comparatively higher.
- Methanol proved to be a more effective solvent for extracting phytochemicals from *Gmelina arborea* leaf meal.
- The methanolic *Gmelina arborea* leaf extract showed higher antioxidant activity than the aqueous extract, except in FRAP, where the aqueous extract performed better.

Recommendation

Based on the findings of this study, the following recommendations are proposed:

- Researchers working with *Gmelina arborea* leaf meal should consider using methanol as the preferred solvent, as it consistently extracted higher levels of bioactive compounds.
- Further investigation using other solvents should be explored to compare their extraction efficiency
- Further studies should examine the biological efficacy of these extracts in live animal
- Toxicity studies should be conducted to determine suitable dosage levels for animals.

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