



# Study on Effect of Solvent on Extraction of Phytochemical Constituents of Red Acalypha (*Acalypha Wilkesiana*) Leaves



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Submission: May 11, 2023; Published: June 06, 2023

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## Abstract

Solvent plays an important role in the medicinal application of any medicinal plant. The medicinal usefulness of any plant solely depends on the solvent-extractable phytochemicals contained in such solvent extract. Hence this research work is aimed in examining the extractive value and qualitative phytochemical screening of extracts gotten from red acalypha (*Acalypha wilkesiana*) leaves using five different solvents. The leaves of red acalypha were obtained, rinsed, cut into smaller pieces, air-dried, ground into powdery sample, and sieved with 40 mm mesh size. The plant sample was extracted using five different solvents (acetone, chloroform, ethyl acetate, methanol, and water) at ratio 1:10 for 72h. Each solvent extract was screened for nine phytochemicals (flavonoid, carotenoid, phenol, oxalate, tannin, saponin, alkaloid, phytate and ascorbic acid). The extractive value of the solvents showed that water had the highest extractive value (13.49±0.34%), followed by methanol (10.92±0.52%), acetone (2.46±0.08%), ethyl acetate (1.48±0.01%) while the least was in chloroform (0.99±0.00%). Qualitative phytochemical screening of the raw *Acalypha wilkesiana* leaves shown that the following seven phytochemicals; flavonoids, carotenoids, phenol, tannin, saponin, phytate and ascorbic acid) were detected out of the nine-phytochemical screened for. Water and methanol extracts contained seven phytochemicals while acetone and ethyl acetate extracts contained one phytochemical and no phytochemical was detected in chloroform extract. Raw leaf sample of red acalypha and its water and methanol extracts contained 77.8%, acetone and ethyl acetate extracts contained 11.1% of phytochemicals screened. Water and methanol are excellent solvents for effective extraction of phytochemicals from red acalypha leaves.

**Keywords:** Red Acalypha; Phytochemicals; Solvents; Extraction; Extractive Value

## Introduction

For many centuries, medicinal plants have been used for therapeutic purposes for the treatment of various diseases [1]. Medicinal plants contain biologically active chemical substances known as phytochemicals such as saponins, tannins, essential oils, flavonoids, alkaloids, and other compounds which have preventive or curative properties [2]. Plant analysis provides chemical information about plants and serves as a guide for their various utilization. Plants leaves have attracted several research interests in food, pharmaceutical, metallurgical, corrosion and other industries [3]. Their usefulness can only be revealed if there are chemical data generated from chemical analysis [4]. Chemical analyses of plant leaves are done to know their proximate, trace metals, heavy metal, mineral, phytochemical, vitamins, and antioxidant [4]. Red acalypha (*Acalypha wilkesiana*) is an ornamental plant common in most gardens and building

surroundings in Nigeria. Its other names include *Acalypha amentacea* and *Acalypha tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can [5]. The Hausas in Northern Nigeria call it "Jiwene" and "Jinwinini", while the Yorubas in South-Western Nigeria call it "aworoso" and it is also available round the year [6]. They are traditionally used in the treatment or management of diverse ailments such as diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, respiratory problems including bronchitis, asthma, and pneumonia as well as skin conditions such as scabies, eczema, and mycoses [7]. However, there is little or no information on the extractive values and the effect of solvents on extractable phytochemicals contained in red acalypha leaves. Consequently, this research focuses on the extractive values and effect of five different solvents on

phytochemical constituents of red acalypha leaves with the view of establishing the most effective solvent(s) in obtaining phytochemicals from raw red acalypha leaves which will be more potent for medicinal therapy.

### Materials and Methods

#### Source of materials

Red acalypha plant was collected from the premises of a residential building in Ondo-City, Ondo State, Nigeria. The plant was authenticated in the Department of Bioscience and Biotechnology, University of Medical Sciences, Ondo- City, Ondo State, Nigeria. All chemicals used were of the analytical grade with the highest purity available (>99.5%) and procured from Sigma Aldrich, USA.

#### Preparation and extraction of red acalypha leaves

Red acalypha leaves were obtained, rinsed in water, cut into smaller pieces for easy drying, air-dried, ground, and finally sieved to give 40 mm mesh size powder. It was put in air-tight containers and kept in a refrigerator at 4°C prior to analysis. The powdered sample was divided into portions, packed in an airtight container labelled appropriately prior to extraction. The sample was extracted separately with each solvent (acetone, chloroform, ethyl acetate, methanol, and water) at ratio 1:10 for 72h during which it was intermittently shaken on a shaking orbit machine. The resulting mixture was filtered through a 0.45µm nylon membrane filter. The extracts were desolventised to dryness under reduced pressure at 40°C by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). Weight of extract obtained was used to calculate the percentage yield (extractive value) of extract in each solvent and the dry extracts were stored in a refrigerator (4 °C) prior to analysis [8-11].

#### Phytochemical analysis of red acalypha leaves and its solvent extracts

The presence of major phytochemical secondary metabolites, namely, saponins, alkaloids, flavonoids, ascorbic acid, phytate, oxalate, tannins, phenolics, and terpenoids were determined using standard phytochemical methods with some modifications.

#### Qualitative determination of phytochemicals of red acalypha leaves and its solvent extracts

##### Test for flavonoids (Cyanidine test)

This was done according to the method of Stankovic [12]. About 0.2g of the plant sample/extract was added with 2mL methanol and 1 mL of concentrated sulphuric acid added. A spatula was used to add a powder of magnesium chloride (MgCl<sub>2</sub>) and the mixture observed for 1 min for effervescence and observed for a brick red coloration.

##### Test for phenol

A small quantity of the extract/ plant sample (about 0.5g) was added to about 0.5mL of FeCl<sub>3</sub> solution. A deep bluish green solution was an indication of the presence of phenol [13].

##### Test for ascorbic acid

Plant samples/extract were crushed in acetic acid and filtered. Few drops of 2, 6-dichlorophenolindophenol solution to the 0.5mL of the filtrate. The presence of faint pink confirmed that ascorbic acid was present [14].

##### Test for saponin

About 0.2g of the extract/plant sample was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy mist of small bubbles) showed the presence of saponin [14].

##### Test for tannin

About 0.2 g of plant sample/extract was stirred with 5mL of distilled water and later filtered. A few drops of FeCl<sub>3</sub> solution were added to 1mL of the filtrate. A blue-black green or blue green precipitate was evidence for the presence of tannin [14].

##### Test for alkaloid (Wagner's test)

This was done according to the method of Joshi [15]. About 0.2g of the plant sample/extract was stirred with 0.4mL of 1%HCl in a water bath for 5 min and filtered. Two grams (2g) of Potassium iodide and 1.27g of iodine were dissolved in 5mL of distilled water and the solution was diluted to 100 mL with distilled water. Two drops of this iodine solution were added to the filtrate; a brown-colored precipitate indicated the presence of alkaloids.

##### Test for oxalate

About 0.5g of sample/extract was boiled with 1mL of 2% H<sub>2</sub>SO<sub>4</sub> solution on water bath. It was filtered while warm and a few drops 1% KMNO<sub>4</sub> was added. The pink color confirms the presence of oxalate [16].

##### Test for phytate

About 0.5 g of the sample/extract was mixed with 2mL of 2% HCl solution. It was filtered and two drops of 0.3% ammonium thiocyanate (NH<sub>4</sub>SCN) solution and 2mL of distilled water were added and shaken. 3 to 4 drops of 10% FeCl<sub>3</sub> solution were then added. Yellow coloration indicates the presence of phytate [16].

##### Test for carotenoids

About 0.5 g of the sample/ extract was mixed with 2mL of distilled water. 5mL of 2% w/v alcoholic KOH solution was added and the mixture was heated on a water bath for 10 minutes. 2mL of chloroform and 0.5g of Na<sub>2</sub>SO<sub>4</sub> were added and shaken thoroughly.

A violet color indicates the presence of carotenoids [16].

**Statistical analysis**

Statistical significance tests were performed using SPSS (v.20, IBM SPSS Statistics, US) at  $p < 0.05$  by means of one-way analysis of variance (ANOVA) followed by LSD post hoc multiple comparison and the experimental results were expressed as mean  $\pm$  standard mean deviation of three replicates.

**Results and Discussion**

The extractive values of red acalypha leaves in different solvents are shown in (Table 1). The five different solvents used are acetone, chloroform, ethyl acetate, methanol, and water. The extractive value of a substance in a solvent is a measure of ability/

capability of a solvent to extract bioactive ingredient/ compound from a substance [17] The extractive values of red acalypha in acetone were  $2.46 \pm 0.08\%$ , chloroform was  $0.99 \pm 0.00\%$ , ethyl acetate was  $1.48 \pm 0.01\%$ , methanol was  $10.92 \pm 0.052\%$  and water was  $13.49 \pm 0.34\%$ . The extractive values of red acalypha in acetone and ethyl acetate were not significant different at  $p < 0.05$ . There was also no significant difference at  $p < 0.05$  for extractive values of red acalypha in methanol and water. The propensity of solvent in extracting phytochemicals from red acalypha was highest in water, followed by methanol, acetone, ethyl acetate and least in chloroform. This suggested that polar solvents were more effective in extracting bioactive constituent in red acalypha leave [18-20].

**Table 1:** Extractive values (% yield) of red acalypha leaves in different solvents

Parameters	Extractive Value (%)
Acetone	2.46b $\pm$ 0.08
Chloroform	0.99a $\pm$ 0.00
Ethyl acetate	1.48b $\pm$ 0.01
Methanol	10.92c $\pm$ 0.52
Water	13.49c $\pm$ 0.34

**NOTE:** Within each row, mean values followed by the same superscript are not significantly different at  $P < 0.05$  level according to Duncan's New Multiple Range Test (DMRT); Values represent means of triplicate determination  $\pm$  standard deviation

The qualitative phytochemical screening of red acalypha leaves and their solvent extracts is presented in Table 2. There were nine phytochemicals screened for red acalypha leaves and their solvent extracts and these bioactive constituents were flavonoids, carotenoids, phenol, oxalate, tannin, saponin, alkaloids, phytate and ascorbic acid. Flavonoids, carotenoid, phenol, tannin, saponin, phytate and ascorbic acid were detected in raw red acalypha leaf and this amount to 77.8% of the phytochemical present in the plant sample. Methanol and water extracts contained flavonoid, phenol, oxalate, tannin, saponin, phytate and ascorbic acid and this gave 77.8% phytochemical detectable. It was only carotenoids

and alkaloids that were not detected in water and methanol extracts of red acalypha leaves. Acetone and ethyl acetate extracts contained only phytates and this resulted in 11.1% of detectable phytochemicals. None of the nine phytochemicals was detected in chloroform extract of red acalypha leaves. It was conspicuously observed that solvents with high polarity had higher detectable phytochemicals than solvents with low polarity. Hence solvents high polarity such as water and methanol served as better solvents in getting extracts with higher medicinal potentials from red acalypha leaves.

**Table 2:** Qualitative phytochemical screening of red acalypha leaves and their solvent extracts.

Parameter	Red acalypha			Solvent extracts		
		Acetone	Chloroform	Ethyl acetate	Methanol	Water
Flavonoids	+	-	-	-	+	+
Carotenoids	+	-	-	-	-	-
Phenol	+	-	-	-	+	+
Oxalate	-	-	-	-	+	+
Tannin	+	-	-	-	+	+
Saponin	+	-	-	-	+	+
Alkaloids	-	-	-	-	-	-
Phytate	+	+	-	+	+	+
Ascorbic acid	+	-	-	-	+	+
%Phytochemical detectable	77.8	11.1	0	11.1	77.8	77.8

+ =Present      - = Absent

## Conclusion

Red acalypha leaves contained essential phytochemicals which were better extracted with methanol and water. Acetone, ethyl acetate and chloroform are poor solvents in extraction of bioactive ingredients from red acalypha leaves. Since majority of phytochemicals detected in red acalypha leaves are also potent antioxidants, then further research can be conducted to investigate the antioxidant activities of methanol and water extracts of red acalypha leaves on edible oils to ascertain their suitability as natural antioxidants over the synthetic ones that are human unfriendly.

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DOI: 10.19080/NFSIJ.2023.12.555830

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