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Extraction, Evaluation, and Antioxidant Activity of Total Phenol from Callus of *Abutilon indicum* (L.) Sweet

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Abstract: In this present study, callus induction from the leaves of *Abutilon indicum* was performed and evaluated antioxidant potential after TLC, PTLC. For induction of callus, Murashige, and Skoog (MS) medium with 2.5 mg/L concentration of 2, 4 Dichloro phenoxy acetic acid (2,4-D) and for assay, Folin Ciocalteau method practiced for evaluation of total phenol, modified spectrophotometer method used for DPPH assay. Based on total phenolic content, total antioxidant capacity, DPPH, assay, and reducing power assay performed after separation and partial purification of total phenols by Thin-layer chromatography (TLC) and Preparative thin-layer chromatography (PTLC), respectively. For total phenol estimation, hot water extract shows the highest total phenol content (10.56 µg/ml) by comparing another extract; similarly, PTLC fraction of callus extract from *Abutilon indicum* shows 61% of inhibition in DPPH at 10 µg/ml. From this study, compared to leaf, callus shows a higher concentration of total phenol and shows promising antioxidant activity. So, it gives scope for further exploration and purification and application of total phenol from callus of *Abutilon indicum.*

Keywords: *Abutilon indicum*; callus; total phenol; antioxidant assay.

1. Introduction

Abutilon indicum (Linn.) sweet is a therapeutic plant, normally known as Thuthi in Tamil /Atibala in Sanskrit. It belongs to the Malvaceae family. It is also called Country Mallow in English and can grow up to 3 feet high [1]. It is an erect, woody, and perennial shrubby plant abundant throughout tropical nations [2]. It is a common roadside weed and is listed among the endangered species of medicinal plants, but it is quite populated in the southern states of Tamil Nadu and Kerala [3]. Customarily, it was accounted for that the plant has therapeutic qualities; for example, leaves are powerful against ulcers and as a fomentation to excruciating pieces of the body[4]. The leaf's decoction is utilized in delicate gums, toothache, and inside for aggravation of bladder. Roots are successful as a demulcent in chest infection, diuretic and urethritis. The bark is utilized as astringent, alexeteric, anthelmintic, febrifuge, and diuretic though seeds are utilized as purgative, expectorant, piles, and so on [5].

All aspects of the plant have therapeutic properties [6]; however, it was found that leaves have more therapeutic properties. In people's medication, *A. indicum* is utilized in treating fever, lung infection, hack, mumps, deafness, menorrhea, hemorrhoids, pee yield, pneumonic tuberculosis, high fever, diabetes, and ringing in the ears [7,8]. Also, previous reports on leaf separates showed antibacterial, hypoglycemic, larvicidal, carminative, hyper-

lipidemic, antipyretic, anti-cough, diuretic, blood tonic, diuretic, and anti-inflammatory properties [9]. The leaf juice is utilized for snappy ulcer mending, easing thirst, loose bowels, gonorrhea, bladder irritation, clean wounds, and ulcers, to treat vaginal diseases, as well as is being utilized as a douche [10]. The plant has adhesive tannins, flavonoids, gallic corrosive, sesquiterpenes, aspergines, phenolic mixes, alkaloids, and saponins [11].

The plant has various properties, such as hypoglycemic, hepatoprotective, antimicrobial, anti-inflammatory, male contraceptive, and anti-diarrheal [12,13]. Phytochemicals such as Phenolics have received greater attention as they are identified as biological response modifiers, and they perform functions such as metal chelators and free radical terminators [14]. The active compounds present in the phenolics have the right structural features for free radical scavenging activity, so they are potent antioxidants [15]. *A.indicum* leaves displayed strong antimicrobial movement against bacterial and parasitic strains test [16]. From the leaf-derived callus of *Abutilon indicum* the phytochemical analysis of bioactive compounds was carried out [17]. Thin-layer chromatography (TLC) and Preparative thin-layer chromatography (PTLC) analysis of an ethanolic extract of callus revealed the presence of total phenol. The spots that coincide with the standard were marked. Spots of samples were scrapped and pooled together and concentrated for further analysis [18]. FRAP is a method used to measure the total reducing power of the electron donors, and it was performed by a modified method described [19].

In FRAP assay, the antioxidant capacity of compounds is determined by the reducing power of the biological material(antioxidant) on reaction with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and to produce a blue colored complex of ferrous tripyridyltriazine (Fe^{2+}-TPTZ). Using DPPH assay, the free radical scavenging effect was assessed as described. DPPH assay is one of the most sensitive antioxidant testing assays, and it is independent of the substrate polarity [20]. It relies on the measurement of antioxidant ability to scavenge the DPPH radical. It is a stable nitrogen-centered free radical, which is violet in color in ethanol solution. It reacts with suitable reducing agents and loses color depending upon the number of electrons taken up. The potency of the antioxidant compound increases with a rapid decrease in absorbance. Total antioxidant capacity was evaluated by using phosphomolybdenum method [21]. This work aims to identify a better source and estimate the antioxidant activity of total phenolic compounds from induced callus of *Abutilon indicum.*

2. Materials and Methods

2.1. Plant sample collection and authentication.

The plant *Abutilon indicum* was collected from Saravanampatti, Coimbatore - 641 035, Tamil Nadu, India, and the same plant was identified as *Abutilon indicum* (L.) Sweet - Malvaceae. by Botanical Survey of India (BSI), Southern Regional Centre, TNAU Campus, Coimbatore - 641 003. Reference No. BSI/SRC/5/23/2018/Tech / 1168.

2.2. Induction of callus and preparation of extract.

The leaf samples of *A.indicum* were collected from Saravanampatty, Coimbatore. They were washed with normal and distilled water and are surface sterilized using 1% mercuric chloride. Callus was induced by using MS media with 2 mg 2,4-D. For the preparation of

extracts, both leaf and callus were taken. Each 1g of leaf and callus sample was taken and were crushed by mortar and pestle using hot and cold water and stored for further study.

2.3. Determination of total phenol.

The total phenolic assay was determined using the Folin Ciocalteau method with the following procedures [22]. 20 of all four samples, i.e., hot leaf extract, cold leaf extract, hot callus extract, cold callus extract, each is taken in different tubes, and all the tubes are made up to 1ml with distilled water. To each tube, 0.5 ml of Folin's reagent and 2.5 ml of 20% sodium carbonate were added. The tubes were then kept in the dark for 40 mins. Then the absorbance value was estimated at 725 nm. The concentration can be determined now using the standard graph of gallic acid.

2.4. Identification and separation of total phenols by TLC and PTLC.

The thin-layer chromatography method was followed by [23] with slight modifications. TLC identified flavonoids present in the crude extract performed under the following conditions: adsorbent layer over the glass plate was silica gel 60, the thickness of the layer was about 0.25 mm, size of the layer was about 20×10 cm, and the glass chamber was about 25×25×14 cm, solvent mixture was taken in an appropriate ratio. Similarly, PTLC was also carried out in glass plates where the procedure is similar to TLC, which was explained by [24].

2.5. Evaluation of the antioxidant potential of total phenol.

2.5.1. Total antioxidant capacity assay.

The total antioxidant capacity assay is based on the principle of reduction of molybdenum (VI) to molybdenum(V) by the extracts and subsequent formations of green phosphate/molybdenum complex at acid pH 0.1 ml of the extracts at different concentrations [25].

2.5.2. DPPH radical scavenging activity assay.

This method was measured by the modified spectrometer method. The principle of this method is that DPPH radical is scavenged by antioxidants through the donation of protons forming reduced DPPH. The solution loses color depending on the number of electrons taken up. The color changes from purple to yellow after reduction, and the antioxidant activity is determined by the decrease of absorbance at 517 nm [26].

2.5.3. Reducing antioxidant power assay.

The reducing antioxidant power assay method is used to determine the antioxidant property present in the samples, which is based on the principle that when the substance reacts with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form a ferric ferrous complex that has an absorption maximum at 700 nm [27].

2.6. Statistical analysis.

All the experiments were triplicated, and standard deviations were represented in that all data and experimental results.

3. Results and Discussion

3.1. Callus induction.

The explants (leaves) were cultured on the media slants to induce the callus using the formulations mentioned above. Initiation of callus was observed after one week of culture, as shown in Figure 1. Callus was produced from the entire cut end of leaf explants after 2 weeks of culture, which was reported in the article [10].

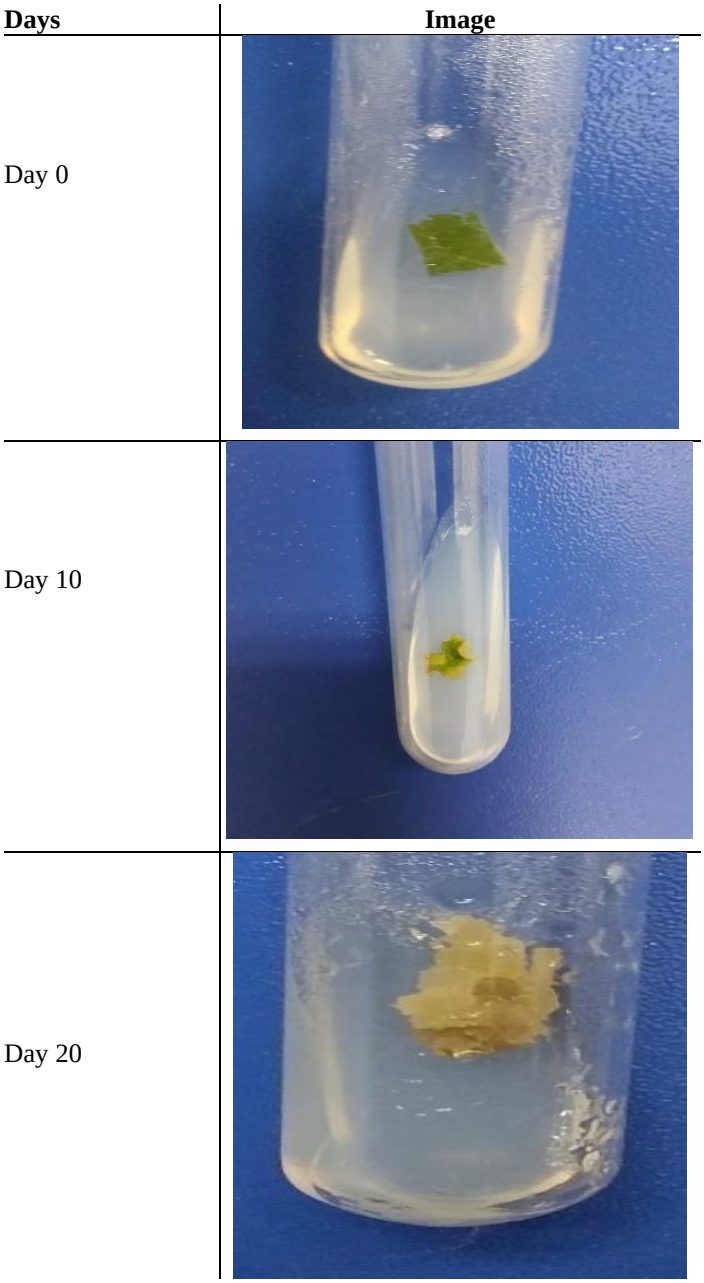

Figure 1. Callus induction from *Abutilon indicum* leaf explant by using MS media.

3.2. Estimation of total phenol.

Figure 2 shows, total phenol content for leaf and leaf derived callus of *Abutilon indicum* using water as a solvent; phenolic content was measured using gallic acid as the standard graph. It has been reported that phytochemical examination showed that alkaloids, phenolics, carbohydrates, protein, and amino acids were present in the methanolic extract [28]. In the study reported in [10]. Has shown that phytochemical screening of methanolic extract of leaf

and leaf derived callus revealed that both *Abutilon* leaves and leaf derived callus possess alkaloids, flavonoids, tannins, and phenolic compounds.

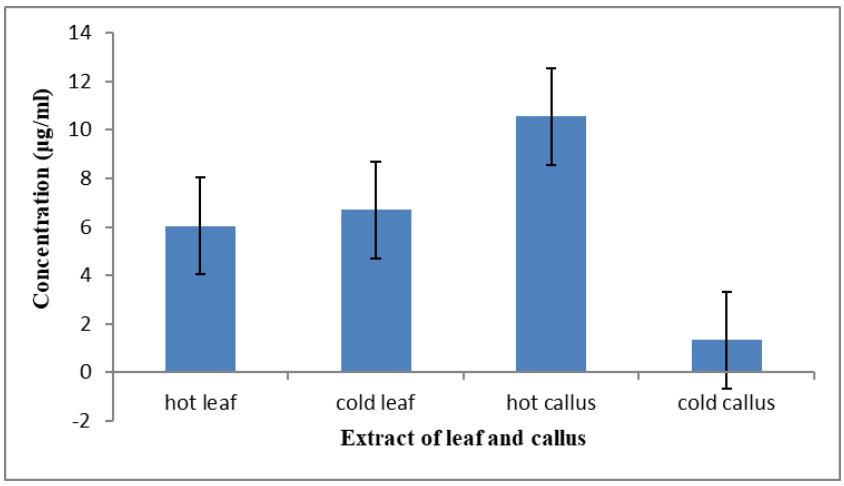

Figure 2. Total phenolic content of leaf and callus extracts.

3.3. Separation and identification of total phenol from callus extract.

Figure 3 revealed the presence of total phenol from TLC plate under UV chamber. After confirming total phenol, extracts separated total phenol by using PTLC and bulk extraction of total phenol carried out from the PTLC silica plate. The final fraction from PTLC, was lyophilized and stored for further study.

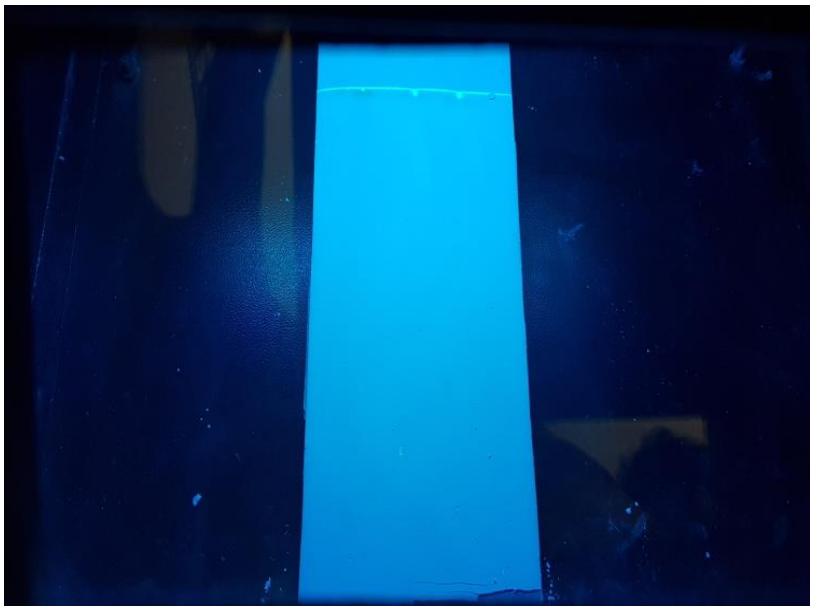

Figure 3. Fluorescent spots of total phenol in TLC under UV illumination.

3.4. Total antioxidant capacity assay.

Antioxidants are substances that can prevent or slow damage to the cells caused by free radicals. Total antioxidant capacity is the general antioxidant assay used to determine antioxidants present in the sample extracts. The antioxidant activity was reported for few chemical compounds in the article [29]. Here the sample extracts with varying concentrations were compared to the standard values of ascorbic acid, and it has been found that with increasing concentrations, the equivalence of ascorbic acid also increased, as shown in Figure

4. From figure 4, for example, for 500 (µg/ml) concentration of about 1 micromoles equivalence of ascorbic acid was noticed in the lyophilized sample of the callus extracts.

3.5. DPPH radical scavenging activity assay.

Since DPPH assay is the most sensitive assay for determining the antioxidant property, the sample extracts with different concentrations were taken, and the assay performed. The standard DPPH assay always uses methanol or ethanol as a solvent. In this assay, Trolox equivalency is used as a standard value for determining the presence of antioxidants. Trolox equivalency is most often measured using the ABTS decolorization assay. The absorbance values were recorded at 517 nm with varying concentrations of the sample extracts. The scavenging activity was checked for various plant extracts compared with butyl hydroxyanisole (BHA) as a standard reported in the article [30].

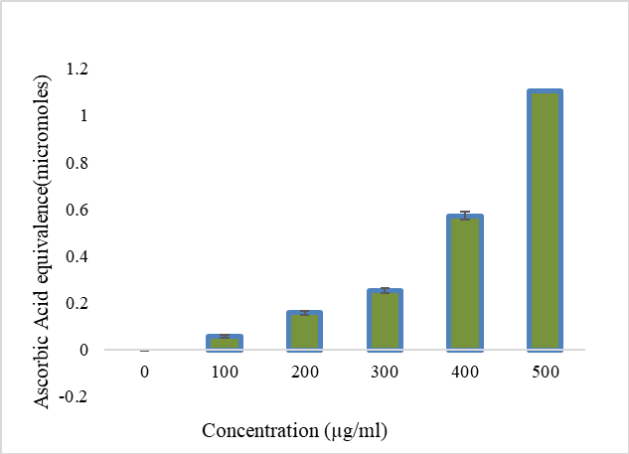

Figure 4. Total antioxidant capacity of total phenol from callus of *A.indicum.*

From figure 5, for example, 40 (µg/ml) concentration about 6 micromoles of Trolox equivalency was noticed. Thus the presence of antioxidant properties in the sample extracts was determined through DPPH assay.

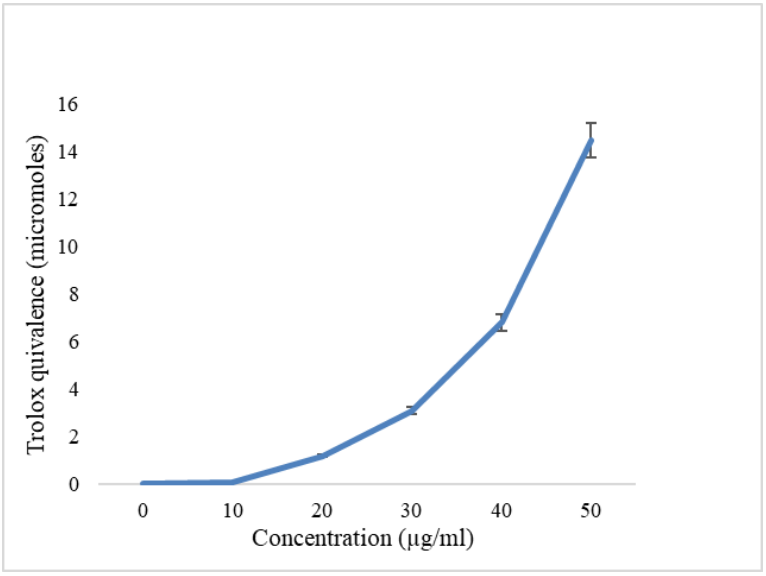

Figure 5. DPPH radical scavenging activity of total phenol from callus of *A.indicum.*

Also, through DPPH assay, the presence of antioxidants was confirmed based on the IC50 principle. Also, the % inhibition in DPPH assay for the chemical compounds were reported in the study made by [29] has shown for different concentrations of ethyl acetate, the % inhibition of the free radicals was found to be 81%. Here the % inhibition was found to be 67%. This means that the sample extracts of the callus can inhibit 67% of the free radicals, which are said to have antioxidant properties. From figure 6, the amount of DPPH radical decreased. On increasing the concentration, the percentage inhibition increased from 60.02 to 70, following the IC50 principle. The data obtained revealed that extracts are free radical inhibitors and thus contain a potent antioxidant.

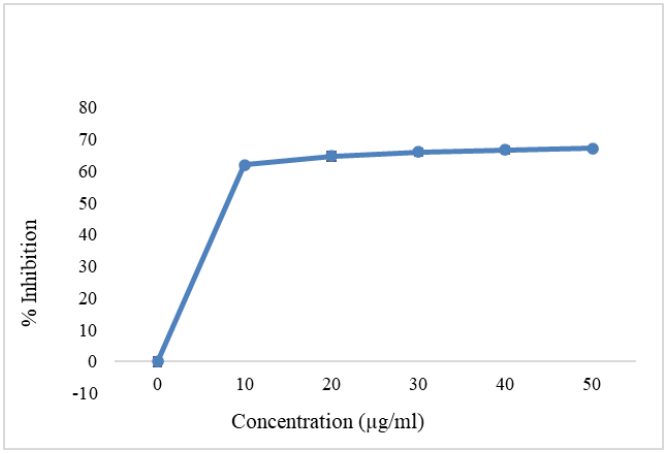

Figure 6. Percentage inhibition of the DPPH radicals due to antioxidant property.

3.6. Reducing antioxidant power assay.

Reducing antioxidant power assay is a widely used method for determining antioxidant molecules present in the sample where antioxidants are used as reductants in a redox-linked colorimetric reaction. The study revealed that the antioxidant property of various fruits peel, pulp, and seed fractions had been reported in the study made by [31]. The study reported here is the most comprehensive comparison of the antioxidant activity among different fruit fractions. Some fruit peel and seed fractions have strong antioxidant activity and may be rich sources of antioxidants. Similarly, here the sample extract concentration varies from 10 to 50 (µg/ml)**,** the absorbance values are seemed to be increased.

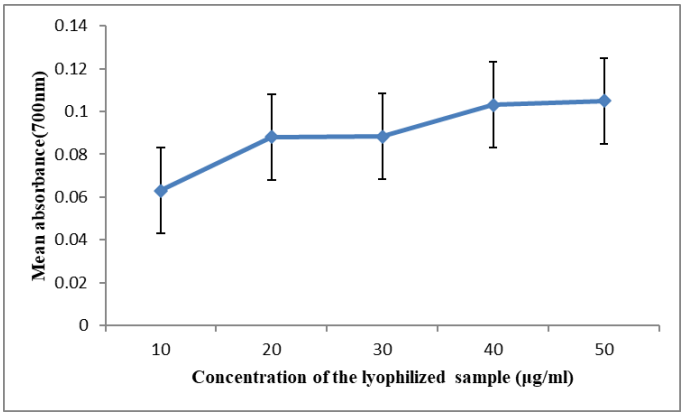

Figure 7. Reducing antioxidant power assay of total phenol from callus.

From Figure 7. the absorbance value increases with an increase in concentration. Thus, reducing antioxidant power assay shows that the antioxidant activity is present in the lyophilized extract of the callus biomass.

4. Conclusions

Initially, the induction of callus formation depends on plant species and which tissues are available for explant culture. An exogenous supply of hormone is required, even using specific media like MS. After callus induction, the extraction and quantification of total phenol from the induced callus and leaves of *Abutilon indicum* phytochemical assays such as phenolic assay was done with the hot and cold leaf extracts, and the comparative studies were done. Ethanolic extracts of leaf-derived callus were then subjected to TLC, and PTLC was carried out. A fraction from PTLC and based on total phenolic content of callus extract, total antioxidant capacity, DPPH, assay, and reducing power assay performed. Finally, we concluded that, compared to leaf, callus shows a higher concentration of total phenol and shows promising antioxidant activity. So, it gives scope for further exploration and purification and application of total phenol from callus *Abutilon indicum.*

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Conflicts of Interest

The authors declare no conflict of interest.

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