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ESTIMATION OF TOTAL POLYPHENOLIC CONTENT IN AQUEOUS AND METHANOLIC EXTRACTS FROM THE BARK OF *ACACIA NILOTICA*

Mayank Tenguria*, Priyankar Chand and Ravi Upadhyay

Vibhav Institute for Research & Application of Biotech, 114/9A, Saket Nagar, Bhopal-462024, Madhya Pradesh, India

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Correspondence to Author:

Mayank Tenguria

Vibhav Institute for Research & Application of Biotech, 114/9A, Saket Nagar, Bhopal-462024, Madhya Pradesh, India

E-mail: biotech_mayank@yahoo.com

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ABSTRACT

Plant polyphenols have been studied largely because of the possibility that they might underlie the protective effects afforded by fruit and vegetable intake against cancer and other chronic diseases. The objective of the present study is to estimate the total polyphenolic content in aqueous and methanolic extract prepared from the bark of *Acacia nilotica* plant species. *Acacia nilotica* (L.) Willd. ex Del commonly known as babul, kikar or Indian gum Arabic tree, has been recognized worldwide as a multipurpose tree. Mostly it occurs as an isolated tree and rarely found in patches to a limited extent in forests and has been widely planted on farms throughout the plains of the Indian subcontinent. The bark, root, gum, leaves and flowers have found use for skin diseases, diarrhoea, dysentery, cough, diabetes, eczema, wound healing, burning sensation and as an astringent, demulcent, anti-asthmatic. For present work the bark of well identified *A. nilotica* (L) plant for the extraction of phytochemicals was done from the village Khargawali (Hoshangabad district). 20-20 grams of the fine powdered sample were subjected to soxhlet extraction with 200 ml distilled water and 40% methanol separately at 70^{o}C and 50^{o}C respectively, for 24 hours and concentrated. The percentage yield so obtained was 35 and 32.5% respectively. The extracts were then subjected for preliminary phytochemical screening of alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides the results of which refers to data given in this article. The total phenolic content of the extracts was determined using the Folin-Ciocalteu method with suitable modification. When compared with the gallic acid standard curve the aqueous extract of 0.01 mg/ml dilution contains 0.323 mg/ml approx of total polyphenol content and that of the methanolic extract of the same concentration shows 0.422 mg/ml approx concentration of TPC. Thus from the present study it can be concluded that the aqueous and methanolic extracts *A. nilotica* are rich in TPC, the potential of which could be utilized in many ways after further advance studies and proper data generation for the development of new chemotherapeutic agents.

INTRODUCTION:

Polyphenols are molecules from the plant kingdom that represent a wide range of substances with various structures[1]. The basic structure is composed of a benzene ring linked to one or more hydroxyl ion, free or involved in another chemical function (e.g. dimethyl ether, ester, sugar). Polyphenols are aromatic compounds formed from the metabolism of shikimic acid and / or that of a polyacetyl. Structurally, they fall into different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols [1, 2].

Plant polyphenols have been studied largely because of the possibility that they might underlie the protective effects afforded by fruit and vegetable intake against cancer and other chronic diseases[3]. The objective of the present study is to estimate the total polyphenolic content in aqueous and methanolic extract prepared from the bark of *Acacia nilotica* plant species.

Acacia nilotica (L.) Willd. ex Del commonly known as babul, kikar or Indian gum Arabic tree, has been recognized worldwide as a multipurpose tree [4]. *A. nilotica* is naturally widespread in the drier areas of Africa, from Senegal to Egypt and down to South Africa, and in Asia from Arabia eastward to India, Burma and Sri Lanka. The largest tracts are found in Sind. It is distributed throughout the greater part of India in forest areas, roadsides, farmlands, tank foreshores, agricultural fields, village grazing lands, wastelands, bunds, along the national highways and railway lines. Mostly it occurs as an isolated tree and rarely found in patches to a limited extent in forests and has been widely planted on farms throughout the plains of the Indian subcontinent [5].

The bark, root, gum, leaves and flowers have found use for skin diseases, diarrhoea, dysentery, cough, diabetes, eczema, wound healing, burning sensation and as an astringent, demulcent, anti-asthmatic. The tender twigs are used as toothbrushes. It has been reported that various parts of the plant are rich in tannins (ellagic acid, gallic acid, tannic acid), stearic acid, vitamin-C (ascorbic acid), carotene, crude protein, crude fibre, arabin, calcium, magnesium and selenium [6, 7, 8].

MATERIALS & METHODS:

Sampling and Extraction: The bark of well identified *A. nilotica* (L) plant for the extraction of phytochemical was done from the village Khargawali (Hoshangabad district). The bark was then treated to remove the dust and then grounded into a fine powder in a mixer grinder. 20-20 grams of the sample powder were then placed separately into the thimble of 250 soxhlet apparatus (Borosil). One was extracted with 200 ml distilled water and other with 40% methanol (Qualigens) at 70^{o}C and 50^{o}C respectively, for 24 hours. The extracts were then subjected to concentration in hot water bath at boiling temperature till the complete evaporation of solvent and stored in refrigerator. The yield was calculated using the following expression:

[Percentage yield = wt. of extract/ wt. of plant material used for extraction X 100]

Preliminary Phytochemical Screening: A small portion of the dry extracts was subjected to the phytochemical test using Harbourne's (1983)[9] methods to test for alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides.

1. **Test for alkaloids:** About 0.2 g extract warmed with 2% H_2SO_4 for two minutes, filtered and few drops of Dragendorff's reagent added orange red precipitate indicates the presence of alkaloids.

2. **Test for tannins:** Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

3. **Test for terpenoids:** About 0.2 g extracts were mixed with 2ml chloroform ($CHCl_3$) and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

4. **Test for saponins:** About 0.2g of the extracts shaken with 5ml of distilled water and then heated to boiling froth (appearance of a creamy mix of small bubbles) shows the presence of saponins.

5. **Test for flavonoids:** Extract of about 0.2 g dissolved in diluted NaOH and HCl added. A yellow solution

that turns colourless indicates the presence of flavonoids.

6. **Test for glycosides:** The extracts hydrolysed with HCl solutions and neutralized with NaOH solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of a glycoside

Estimation of TPC: The total phenolic content of the extracts was determined using to the Folin-Ciocalteu method [10] with suitable modification. The extracts were suitably diluted with their respective solvents and oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue colour was measured at 750 nm after 60 min. Using gallic acid as standard total phenolic content (standard curve was prepared using concentrations 0-500 mg/L) was expressed as mg GA equivalent/L of extract. Data reported of three replications.

RESULTS & DISCUSSION: The yield of aqueous and methanolic extracts from the bark of *A. nilotica* was 35 and 32.5% respectively. The preliminary phytochemical analysis of these extracts for the presence of alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides are being depicted in **table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF ACACIA NILOTICA BARK EXTRACTS

Constitutents	Aq Extract	Methanolic Extract
Alkaloids,	+++	+
Tannins	+	+++
Terpenoids	+++	+
Saponins,	++	-
Flavonoids	++	-
Glycosides	--	--

[(+) means present, (++) means Prominent, (+++) means more prominent and (-) means absent]

The diluted extracts of 0.01 mg/ml were used in estimation of TPC to successfully perform the reaction with FC-reagent. When compared with the gallic acid standard curve (**fig. 1**) the aqueous extract of 0.01 mg/ml dilution contains 0.323 mg/ml approx of total polyphenol content and that of the methanolic extract of the same concentration shows 0.422 mg/ml approx concentration of TPC.

The Folin-Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols[11]. From the results obtained it is clear that the aqueus extract of bark of *A.nilotica* is more rich in number of phytochemical than methanolic extract but the results of Folin-Ciocalteu assay, the total phenolic content of methanolic extrat is higher among both.

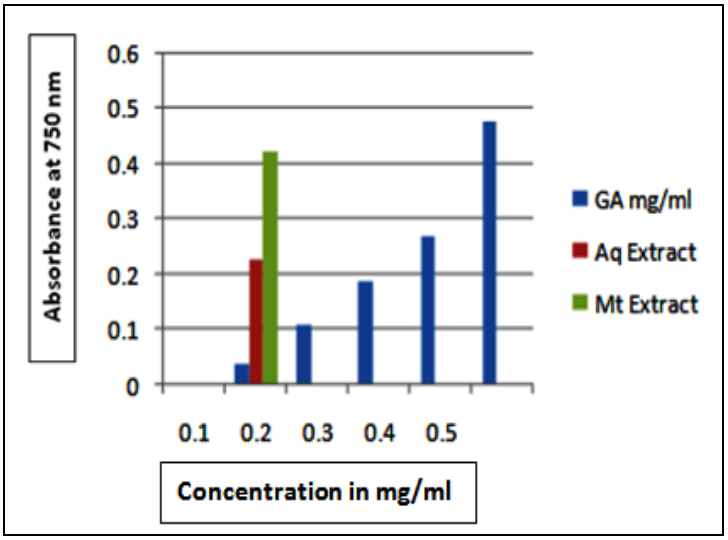

FIGURE 1: STANDARD BOX PLOT AND ITS COMPARISON WITH AQ AND METHANOLIC BARK EXTRACTS OF *A. NILOTICA*

It is well known that plant phytochemicals/phenolics, in general, are highly effective free radical scavengers and antioxidents. Hydrogen donating property of the polyphenolic compounds is responsible for the inhibition of free radical induced lipid peroxidation [12]. Consequently the antioxidant activities of plant/ herb extracts are often explained with respect to their total phenolic and flavonoid contents, with good correlation [13].

CONCLUSIONS: Plants are the important source of potentially useful structures for the development of new chemotherapeutic agents [14]. Here in present study, extracts obtained from the bark of *Acacia nilotica* are rich in total phenolic contents. From the results of preliminary tests and TPC estimation, the aqueous extract is richer in number of phytochmecals present than that of the methanolic extract though its TPC value is higher than aqueous extract. Thus from the present study it can be concluded that the aqueous and methanolic extracts of *A. nilotica* are rich in TPC the potential of which could be utilized in many ways after further advance studies and proper data generation for the development of new chemotherapeutic agents.

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