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Phytochemical screening and free radical scavenging Activity of "*Amrutha Mahabala Kashayam*"- an ethnobotanical formulation

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Abstract

In this article, we have investigated preliminary phytochemical screening and antioxidant ability for the aqueous extract of "*Amrutha Mahabala Kashayam*" (AMK) an ethnobotanical formulation prescribed around Kuppam forest habitats in Andhra Pradesh, India. Quantitative estimation of phytoconstituents and DPPH, ABTS assay were performed for examining Antiradical scavenging potency. Results depicted aqueous extract of AMK possess High contents of phytochemical compounds (TPC, TFC, TF and TPA) and exert strong anti-radical scavenging capacity by scavenging 64.22 % of DPPH radicals and 58.24 of ABTS % radicals at 100 mg/mL and exhibited IC_{50} values of DPPH and ABTS of the aqueous extract of AMK are 62.12±1.33 µg/ml and 72.62±2.03 µg/ml respectively. Our results reveals that, the AMK proves to be a potent Neutraceutical and provided a scientific base for a therapeutic ethno-pharmacological insight, authenticating their usages in folk medicine.

Key Words: Phytochemicals, free radicals, scavenging Activity, Amrutha Mahabala Kashayam, Ethnobotanical formulation.

Introduction

Awareness of medicinal plants usage is a result of the many years of struggles against diseases and man learned to pursue drugs in barks, seeds, fruits, and other parts of the plants. (Srivastava, 2018; Suksungworn and Duangsrisai, 2021). The Plant drugs are very much effective in curing diseases with less or no side-effects which is possible due to their antioxidative characters (Oliveira-Neto *et al.*, 2017; Senguptha *et al.*,2018; Leite *et al.*, 2018; Eruygur *et al.*,2019). Antioxidants plays a key role in protecting against oxidative damage. Plant extracts has a great potency in playing as Natural antioxidants (Edziri *et al.*,2020).

In the current scenario of health management, the discovery of therapeutic natural products which was reported on ethnobotanical claims need to be investigated in vitro. (Radwan etal.,2020). This establish the link between traditional, folklore usage and bioactivities for ensuring safety before clinical use. Many tribal formulations prescribed in and around Rayalaseema region of Andhra Pradesh have been documented by previous workers (Bharathamma *et al.*, 2015; Sripriya, 2017 & 2018; Preeti Singh, 2018; Sripriya and Babu, 2019a&b; Sripriya and Sekhara, 2020). Very less documentation of ethnobotanical information on decoctions and its efficacy has not been tested effectively. Previously, we have reported antioxidant activity

of **"*Nitya Yevvana Kashayam*"** an ethnic formulation (Sripriya and Sekhara, 2020) which proved to be a great antiradical scavenger.

In our ethnobotanical survey we have found **"*Amrutha Mahabala Kashayam*"** (AMK), a polyherbal formulated with plant parts in the form of syrup is prescribed for Strength giving, Immunity booster and antimicrobial medicine for the tribal hamlets inhabiting the forests around Kuppam, Andhra Pradesh. Literature survey, Internet resources done on scientific validation found inadequate on this polyherbal decoction. So we intended to test **this "*Amrutha Mahabala Kashayam*"** The objective of present investigation is to estimate phytochemicals and measure the antioxidant capacity present in the AMK

Methodology:

Ethnobotanical Information was gathered from the ethnic healers and tribal women during our survey (2018-2020) around unexplored Kuppam ethnic tribal hamlets. Perusal of literature on documentation and analysis on these ethnic medications is inadequate which are formulated in the form of liquids, syrups. Table 1. Shows the raw material and preparations of the selected drug "*Amrutha Mahabala Kashayam*" as prescribed by the tribal physician since two decades and was procured within a small pot for testing. Proper baseline survey was done according to standard methodologies (Jain and Rao, 1977 and Hemadri *et al.*,1978a&b; Jain, 1981) and information was compared with other latest research literature Sudarsanam *et al.*,2019). Plant identification was done according to Gamble and Fischer (1935) and Pullaiah *et al*., (2018).

Methodology adapted for extraction was done according to the protocol followed by Edziri *et al.,* (2020); Cosme et al., (2020). Preliminary screening of phytoconstituents was performed for screening of alkaloids, saponins, terpenoids, anthraquinones, Cardiac glycosides, coumarins, phlobatannins, tannins, flavonoids, indoles, leucoanthocyanins, steroids, phenols, proteins, lignin using standard methods (Kokate, 1999 and Khandelwal, 2003; Harborne 1973; Trease, and 1989).

Table: 1 Detail information for the contents used for MBK.

Sr.No	Name of the Plant	Family	Part used	Preparation/ Dosage
1	*Aegle marmelos*	Rutaceae	Mature fruit (Mesocarp + Exocarp)	Fresh mature fruits collected and after removing seed squeezed and soaked in one litre water
2	*Terminalia arjuna*	Combretaceae	Fresh Bark	1 kg of fresh chopped bark heated in 2 Litre of pure water.
3	*Tinospora cordifolia* Amrita, guduuchii	Menispermiaceae	Leaf	Leaf crushed with Ten grains of black pepper and 3-4 drops of pure honey were mixed together
4	*Ocimum gratissimum* L.,	Lamiaceae	Leaf	Fresh leaves were squeezed and decoction is heated for 10-15 minutes and the mixed with all ingredients

Quantitative analysis of Phytoconstituents:

Quantitative analysis Ash, Soluble carbohydrates, Crude fiber, Starch, Moisture, Proteins was performed using standard method (Horowitz, 2000; AOAC, 2005). Carbohydrates were quantified by the method of Pons *et al*. (1981). Total Fatty composition was determined using anthrone method of Blight and Dyer (1959). The Extraction yield also determined [13]. Quantitative analysis soluble carbohydrates, Starch, Crude fiber, Proteins, Moisture, Ash was performed using standard method employed by Horowitz (2000).

Quantitative analysis of antioxidants:

Total phenolic content (TPC) was determined by employing Folin–Ciocalteu assay as per Oliveira-Neto *et al.*, (2017) with certain modifications. Total flavonoid content (TFC) was measured using standard colorimetric assay (Karthikeyan and Vidya, 2019). Total flavonols (TF) in the plant extracts were estimated as per the method employed by Kumaran and Karunakaran (2007). Determination of total proanthocyanidins (TPA) was carried by employing standard calorimetric method reported by Sun *et al*. (1998). Estimation of Lipids by Bligh and Dyer (1959) Carbohydrates by Prosky *et al.* (1984) Condensed Tannins was evaluated by vanillin assay as described by Bi Athom *et al*. (2018)

In vitro Antioxidant assays:

The scavenging activity of DPPH was assessed by scavenging of 2, 2-diphenyl-1-picrylhydrazyl radicals in accordance with the Traditional method of Brand Williams et al., (1995); Oliveira-Neto *et al.*, (2016). Follwded according to the modified protocol of Mitta *et al.*, (2014) and Nath et al., (2013). ABTS (Azino-bis 3-ethylbanzthiazoline-6-sulphonic acid) radicals scavenging activity was evaluated by following the standard Protocol followed by Re R *et al.* (1999) Absorbance at 517nm (For DPPH Assay), 734 nm (For ABTS Assay) was monitored with a UV-Vis spectrophotometer. Reduction of phosphomolybdenum was calculated to determine the total antioxidant capacity (TAC) by adapting the method of Umamaheswari and Chatterjee and Prieto *et al.*, (1999).

The aqueous extract was prepared in a series of concentrations (25, 50, 100 mg/mL). The amount of extract in g/ml required to reduce the DPPH absorbance by 50% (IC_{50}) was calculated in order to evaluate the antioxidant capacity of each sample, whereas the percentage of discoloration express the free radical scavenging activity and is calculated with this equation:

The both experiments were carried out in triplicate. The % inhibition for DPPH and ABTS assay was calculated according to the formula:

Antioxidant activity (%) =

$$\left\{ \frac{\mathbf{ABS}_{\mathbf{DPPH/ABTS}} - \mathbf{ABS}_{\mathbf{sample}}}{\mathbf{ABS}_{\mathbf{DPPH/ABTS}}} \times \mathbf{100} \right\}$$

ABS- Absorbance; IC_{50} **= (Concentration of test / 50% nearest FRSA) × 50;** The IC_{50} value calculated as: IC_{50} value (mg/mL)

IC_{50} is the amount of extract in g/ml of the tested samples required to decrease the initial concentration of DPPH, ABST by 50%(Leite *et al* 2018).

RESULTS AND DISCUSSION

Evaluation of ethnobotanical claim employed in folk medicine found to be an useful and interesting task, predominantly for finding inventive source of natural antioxidants, functional foods, and nutraceuticals. In this research, we have evaluated AMK preliminary phytochemical screening as well as its antioxidant capacity.

Qualitative analysis:

Qualitative analysis of phytochemicals indicated the presence of Alkaloids, Flavonoids, Phenols, Carbohydrates, Proteins, Lignin's, Steroids and however, saponins, starch, Phytosterols, reducing sugars, Indoles, Leucoanthocyanins, fixed oils and Fats and Gum were absent (Table 2).

Table 2: Preliminary phytochemical analysis of AMK.

Name of the test	Amrutha Mahabala Kashayam
Alkaloids	+
Amino acids	++
Anthocyanidins	+
Anthroquinones	+
Carbohydrates	++
Coumarins	+
Flavonoids	++
Glycosides	-
Indoles	-
Leucoanthocyanins	-
Gums	-
Phenols	++
Proteins	++
Phytosterol	-
Quinones	+
Reducing sugars	++
Saponins	+
Steroids	+
Starch	-
Fixed oils and Fats	-
Tannins	+
Terpenoids	+
Lipids	+
Lignins	+
Lignans	+
Indoles	+

Quantitative analysis - Proximate composition:

As the Proximate composition provides a valuable information about its medicinal and nutritional quality (Ullah *et al*., 2018), in this research we acquired a good quantity percentage. Quantities of different phyto-constituents such as soluble carbohydrates, Crude fibre, Proteins, Fat composition, Moisture, Ash found to be 4.33± 2.1, 28.09 ± 1.2, 22.09 ± 3.1, 16.62 ±1.8, 17.42 ± 3.3, 14.33 ± 2.5 % respectively. Extraction yield was 6.34±3.24 % (Table 3). Data presented are in Mean ± Standard error.

Table 3: Proximate estimation of AMK

Sr.No.	Proximate composition	Quantity (%)
1	Crude Protein %	22.09 ± 3.1
2	Moisture content %	17.42 ± 3.3
3	Ash %	14.33 ± 2.5
4	Crude Fat %	16.62 ±1.8
5	Crude Fibre %	28.09 ± 1.2
6	Soluble Carbohydrates	4.33± 2.1
7	Extractive Yield	6.34±3.24

Quantitative analysis of antioxidants:

Antioxidants of aqueous extract such as Phenols, flavanoids, Flavanols and anthocyanidines were quantified are as follows: Total Phenolic Content (TPC, Gallic Acid Equivalent) is 364.09±3.18 mg/g. Total Flavonoid Content (TFC) is 138.21±2.09 mg/g, Quercetin Equivalent). Total Flavanols are 174.12±3.33 mg/g (TF, Catichin Equivalent). Total Proanthocyanidins are 61.09±2.30 mg/g (TPA, Catechin Equivalent).

The in-vitro antioxidant activity was assessed with Two different assays viz., DPPH and ABTS assay. The capacity of the extracts to inhibit the ABTS, DPPH were expressing by estimating IC_{50} value (mg/mL) for both aqueous extracts. Table 4 shows the antioxidant activity in aqueous extract of AMK. The inhibition concentration (IC_{50}) was estimated in the study and was measured as minimal IC_{50} value means maximum anti-oxidant activity (Singleton *et al*.,1999).

The observance of discoloration effect due to the formation of reduced form of DPPH and ABTS measured spectrometrically is the active principle in evaluation of antioxidant activity (Leite *et al* 2018; Aryal *et al*.,2019).). The predicted phytochemicals constituted antioxidant activities when assayed with DPPH and ABTS and compared with synthetic antioxidants Butylated Hydroxy-toluene (BHT).

Here, IC_{50} values of DPPH and ABTS of the aqueous extract of AMK are152.12 ± 1.33 µg/ml and 92.62 ±2.03 µg/ml respectively. The phosphomolybdate method which is quantitative and expressed as Total antioxidant capacity (TAC) is evaluated with ascorbic acid equivalents and the IC_{50} value is 37.34±3.24 µg/ml. (Table 4). Results showed that aqueous extract of AMK had the best antioxidant activity by DPPH and ABTS methods with the lowest IC_{50}, this antioxidant activity was bit higher when compared to the positive control. The results pertaining to anti-oxidant activity are presented in Table 3. In DPPH assay The

IC_{50} value of standard was 29.44±0.78 µg/ml of ascorbic acid equivalent/mg. The results of the study indicated that the AMK at 100mg/mL found to be equivalent to the standard indicating that AMK at 100mg/mL substitutes synthetic antioxidant BHA. In ABTS assay, IC_{50} acquired 31.16±1.14 µg/ml ascorbic acid equivalents per mg of sample.

The percent deterrence of DPPH radical by the extracts was compared to a known synthetic antioxidant, Butylated Hydroxy-toluene (BHT). Colour density shows the anti-oxidant potency of the extract. (Bhatt and Negi, 2012) The per cent inhibition was calculated by measuring the absorbance of extract/BHT treated samples against the blank. The IC50 values for the aqueous extract were calculated and compared with the standard reference compound ascorbic acid (Yen et al.,2002),

Table 3: Antioxidant activity of AMK

Sample tested	Conc. (µg/mL)	(DPPH radicals scavenged) % Inhibition ± SD	(ABTS radicals scavenged) % Inhibition± SD	TAC (µg/mL)
AMK	25	09.33±1.64 %	12.36±0.68 %	20±3.2 (µg/mL)
	50	21.21±0.90 %	26.18±1.66 %	52± 4.4 (µg/mL)
	100	54.18±0.44 %	63.48±1.60 %	75±1. 8 (µg/mL)
IC_{50} AMK	-	29.44±0.78 (µg/mL)	31.16±1.14 (µg/mL)	-

Tinospora cordifolia Miers. (Menispermiaceae) popularly as Amrita, guduuchii has already been proved to be an antioxidant and immunomodulant. (Sinha *et al.*,2004. and Kumar *et al.*,2012). Bark of *Terminalia arjuna* has been a great cardio protector and immunomodulant (Halder *et al.*, 2009). It is unknown that which plant constituents are associated in reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicine.

Our results summarized and depicted that high concentrations of natural functional phytoconstitutens and antioxidants which are therapeutic in nature were present proving that the "MBK" in decoction form can be consumed or can be utilized as phytomedicated and was also subjected to test for various constituents.

Conclusion:

AMK an ethnobotanical syrup containing valid amount of high valued phytocompounds (Phenols, Flavonoids, Flavanols) and bio compounds which play as a potential natural source of antioxidants.

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