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QUALITATIVE AND QUANTITATIVE ANALYSIS, ANTI-OXIDANT ACTIVITY OF SIDDHA FORMULATION *KATTU MAANTHA KUDINEER*

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ABSTRACT

Siddha system of medicine is a traditional system of Indian medicine. In Siddha medicines herbal formulations have no side effects and some herbs naturally have anti-oxidant property. To prove scientifically qualitative and quantitative analysis carried out to prove the active constituents. *Kattu maantha kudineer* study results proves it contain anti-oxidant activity by using DPPH assay, physicochemical, biochemical analysis and determination of total phenolic content were carried out. The study results also proves the presence of iron, zinc, calcium, starch, reducing sugar.

KEYWORDS

Siddha, *Kattu maantha kudineer*, Physicochemical, Anti-oxidant activity, DPPH assay and Phenolic content.

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INTRODUCTION

The term siddha denotes perfection. It is orginated from southern part of Tamilnadu more than 3000 years ago. In siddha literature *kattu maantham* is one of the twenty one types of *maantham* that occurs in children. *Kattu maantha kudineer* was prescribed for *kattu maantham* to childerns below 12 years. Qualitative and quantitative analysis carried out to prove the active constituents of *Kattu maantha* kudineer[1]. Using DPPH assay scavenging method anti-oxidant activity and total phenolic content of *Kattu maantha kudineer* was calculated. Toxic constituents like lead, arsenic, mercury were absent. *Kattu maantha kudineer* contain ten different herbs in which *Vitex negundo* prove the

presence of anti-flatulent activity[2]. *Phyla nodiflora, Pergularia daemia*, *Azadirachta indica, Allium sativum, Piper longum, Carum copticum* are used in indigestion, fever, diarrhea, internal piles, cough by siddhars for numerous years. *Mangnifera indica, Vitex negundo, Azadirachta indica* has proved to have anti-oxidant, immunomodulation, hepatoprotective activity[3]. *Morinda tinctoria* represented as noni has proved to possess anti-cancer,anti-bacterial, anti-oxidant, anti-hypertensive, immune enhancing, anti -viral, anti-inflammatory effects[4.]

MATERIAL AND METHODS

Sop of *kattu maantha* kudineer

Kattu maantha kudineer is a herbal siddha formulation comprising of ten different types of herbs like Poduthalai erkku *(phyla nodiflora)* Maa elai erkku *(Mangnifera indica),* Puliyam erkku *(Tamarindus indica),* vembuerkku *(Azadirachta indica),* Nuna erkku *(Morinda tinctoria)*, Veliparuthi erkku (*Pergularia daemia),* Nochi erkku *(Vitex negundo), Poondu (Allium sativum),* Tippili *(Piper longum), Omam* (*Carum copticum).* The raw drugs were identified and authenticated by the botony department in siddha central research institute Arumbakkam, Chennai. The purified raw drugs are made into course powder. The trial drug *Kattu maantha kudineer* is stored in clean dry air tight container and is dispensed to patients in pockets.

Biochemical analysis

Preparation of Sodium Carbonate Extract

2gm of the sample *Kattu Maantha Kudineer,* is mixed with 5gm of Sodium Carbonate and taken in a 100ml beaker and 20ml of distilled water is added. The solution is boiled for 10 minutes, cooled and then filtered. The filtrate is called Sodium Carbonate Extract.

Physicochemical analysis

Preparation of the plant extract

Preparation of the extracts was assessed by following method, One gram of dried powder of KMK plant materials were extracted with 20 ml aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Phytochemical Screening of plant Extracts of KMK

The phytochemical screening of palnt extracts KMK were analysed after extraction by three solvents (etheric, ethanolic and aqueous)[5-8] Phytochemical screening was carried out on the plant extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the leaf extracts tested[9].

Phytochemical analysis

Test for Tannins

For tannin identification, 1 ml of plant extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green or a blue-black coloration.

Test for Saponins

For saponin identification, 2ml Plant extract, 2ml of distilled water was added and shaken in graduated cylinder for 15 min lengthwise, formation of 1cm layer of foam indicates the presence of saponins

Test for Quinones

For Quinones identification, 1ml Plant extract, 1ml of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour indicates the presence of Quinones

Test for Flavonoids

For flavonoids identification, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Alkaloids

For Alkaloids identification, 2ml Plant extract, 2ml of concentrated Hydrochloric acid (HCl) was added. Then few drops Mayer's reagent was added**.** Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides

For Glycosides identification, 2ml of the plant extract, 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

Test for Cardiac glycosides

For Cardiac glycosides identification, 0.5 ml of the plant extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

Test for Terpenoids

For Terpenoids identification, 0.5 ml of the plant extract, 2 ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Phenols

For phenol identification, 1ml of the plant extract, 2ml of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue / green colour indicates the presence of phenol

Test for Coumarins

For identification of coumarins 1 ml of plant extract and 1 ml of 10 % NaOH were added. Formation of yellow colour indicates the presence of coumarins.

Test for Steroids

Steroids was sought by Liebermann reaction. 10 ml of ethanolic extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride, we added 0.5 ml of hot acetic anhydride, and added 0.5 ml of filtrate chloroforme. Treated with the reagent, appearance at the interphase, a ring of blue-green shows positive.

Test for Anthocyanin and Beta cyanin

To 2ml of the plant extract, one ml of 2N sodium hydroxide (NaOH) was added and heated for 5 min at 100 °C. Appearance of bluish green colour indicates the presence of anthocyanin and yellow colour indicates the presence of betacyanin

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ANTI-OXIDANT ACTIVITY

Quantitative analysis of free radical scavenging activity of sample **of *Kattu maantha Kudineer* (KMK)**

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of sample extracts of *Kattu maantha Kudineer* (KMK) were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.,* 2005)[11]. Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula[10].

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100

Determination of Total Phenolic Contents in *Kattu maantha Kudineer* (KMK)

Total phenolic content in the aqueous sample extracts of *Kattu maantha Kudineer* (KMK) was determined by the Folin Ciocalteau colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml aliquot of sample was added to 0.5 ml of Folin- Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

RESULTS AND DISCUSSION

S.No	EXPERIMENT	OBSERVATION	INFERENCE
I.	**TEST FOR ACID RADICALS**		
1. a.	**TEST FOR SULPHATE** 2ml of the above prepared extract is taken in a test tube. To this, 2ml of 4%Ammonium Oxalate solution is added.	Absence of White Precipitate	Absent
b.	2ml of the extract is added with 2ml of Dilute Hydrochloric acid until the effervescence ceases off. Then 2ml of Barium Chloride solution is added.	Absence of White Precipitate	Absent
2.	**TEST FOR CHLORIDE** 2ml of the extract is added with Dilute Nitric Acid until the effervescence ceases. Then 2ml of Silver Nitrate solution is added	Absence of White Precipitate	Absent
3.	**TEST FOR PHOSPHATE** 2ml of the extract is treated with 2ml of Ammonium Molybdate solution and 2ml of Concentrated Nitric Acid.	Absence of yellow Precipitate	Absence
4.	**TEST FOR CARBONATE** 2ml of the extract is treated with 2ml of Magnesium Sulphate solution.	Absence of White Precipitate	Absent
5.	**TEST FOR SULPHIDE** 1gm of the substance is treated with 2ml of Concentrated hydrochloric Acid.	Absence of Rotten egg smell	Absent
6. a.	**TEST FOR FLUORIDE AND OXALATE** 2ml of extract is added with 2ml of Dilute Acetic Acid and 2ml of Calcium Chloride solution and heated.	Absence of White Precipitate	Absent
b.	5 drops of clear solution is added with 2ml of dilute Sulphuric Acid and slightly warmed. To this, 1ml of Dilute Potassium Permanganate solution is added.	Absence of Potassium Permanganate solution discolouration	Absent
7.	**TEST FOR BORATE** 2 pinches of the substance is made into a paste by using Sulphuric Acid solution and Alcohol (95%) and introduced into the flame.	Absence of Green tinged flame	Absent
II.	**TEST FOR BASIC RADICALS:**		
8.	**TEST FOR LEAD** 2ml of the extract is added with 2ml of Potassium Iodide solution.	Absence of Yellow precipitate	Absent
9. a.	**TEST FOR COPPER** One pinch of the substance is made into a paste with Concentrated Hydrochloric Acid in a watch glass and introduced into the non-luminous part of the flame.	Absence of Bluish Green coloured flame	Absent
b.	2ml of the extract is added with excess of Ammonia solution.	Absence of deep blue	Absent
10.	**TEST FOR ALUMINIUM** To the 2ml of the extract, Sodium Hydroxide solution is added in drops to excess.	Absence of White Precipitate	Absent

11.	**TEST FOR IRON** To the 2ml of the extract, 2ml of Ammonium Thiocyanate solution and 2ml of Concentrated Nitric acid is added.	Blood red colour is present	Present
12.	**TEST FOR ZINC** To the 2ml of the extract, sodium Hydroxide solution is added in drops to excess.	Absence of White Precipitate	Present
13.	**TEST FOR CALCIUM** 2ml of the extract is added with 2ml of 4% Ammonium Oxalate solution	Absence of White Precipitate	present
14.	**TEST FOR MAGNESIUM** To the 2ml of the extract, Sodium hydroxide solution is added in drops to excess.	Absence of White Precipitate	Absent
15.	**TEST FOR AMMONIUM** To the 2ml of the extract, few ml of Nessler's Reagent and excess of Sodium Hydroxide solution are added.	Absence of Reddish Brown precipitate	Absent
16.	**TEST FOR SODIUM** 2 pinches of the substance is made into a paste by using Hydrochloric Acid and introduced into the blue flame.	Absent of Yellow colour flame	Absent
17.	**TEST FOR MERCURY** 2ml of the extract is treated with 2ml of Sodium Hydroxide solution.	Absence of Yellow precipitate	Absent
18.	**TEST FOR ARSENIC** 2ml of the extract is treated with 2ml of Silver Nitrate solution	Absence of Yellow precipitate	Absent
19.	**TEST FOR STARCH** 2ml of the solution is treated with weak Iodine solution.	Blue colour is obtained	Present
20.	**TEST FOR REDUCING SUGAR** 5ml of Benedict's Qualitative solution is taken in a test tube and allowed to boil for 2 minutes and 10 drops of the extract is added and again boiled for 2 minutes. The colour changes are noted.	Green colour is obtained	Present

CHEMICAL ANALYSIS OF TRIAL MEDICINE – *KATTU MAANTHA KUDINEER*

S.No	CONSTITUENTS	*KATTU MAANTHA KUDINEER*
ACID RADICALS		
1.	SULPHATE	ABSENT
2.	CHLORIDE	ABSENT
3.	PHOSPHATE	ABSENT
4.	CARBONATE	ABSENT
5.	SULPHIDE	ABSENT
6.	FLURIDE AND OXALATE	ABSENT
7.	BORATE	ABSENT
BASIC RADICALS		
8.	LEAD	ABSENT
9.	COPPER	ABSENT
10.	ALUMINIUM	ABSENT

11.	IRON	PRESENT
12.	ZINC	PRESENT
13.	CALCIUM	PRESENT
14.	MAGNESIUM	ABSENT
15.	AMMONIUM	ABSENT
16.	SODIUM	ABSENT
17.	MERCURY	ABSENT
18.	ARSENIC	ABSENT
19.	STARCH	PRESENT
20.	REDUCING SUGAR	PRESENT

The biochemical analysis of *Kattu Maantha Kudineer* shows the presence of Iron, Zinc, Calcium, Starch and reducing sugar.

Table No.1: Phytochemical screening from plant extracts of KMK

S.No	Phytochemicals Tested	plant extracts of *K*MK
		Aqueous
1	Tannins	++
2	Saponins	++
3	Quinones	++
4	Terpenoids	++
5	Steroids	++
6	Flavonoids	++
7	Phenol	++
8	Alkaloids	-
9	Glycosides	-
10	Cardiac glycosides	+
11	Coumarins	++
12	Antho cyanin	-
13	Beta cyanin	+

Key: + = positive, ++ = strong positive , - = negative

It was noticed that tannins, saponins, quinones, terpenoids, steroids, flavonoids, phenol, coumarins were found in extract of *Kattu Maantha Kudineer.*

Physiochemical analysis OF KMK

S.No	Sample	pH	Moisture (%)	Ash (%)	Cadmium	Crude fibre
1	KMK	5.7	1.3	32.21	negative	30.42

ANTI - OXIDANT ACTIVITY

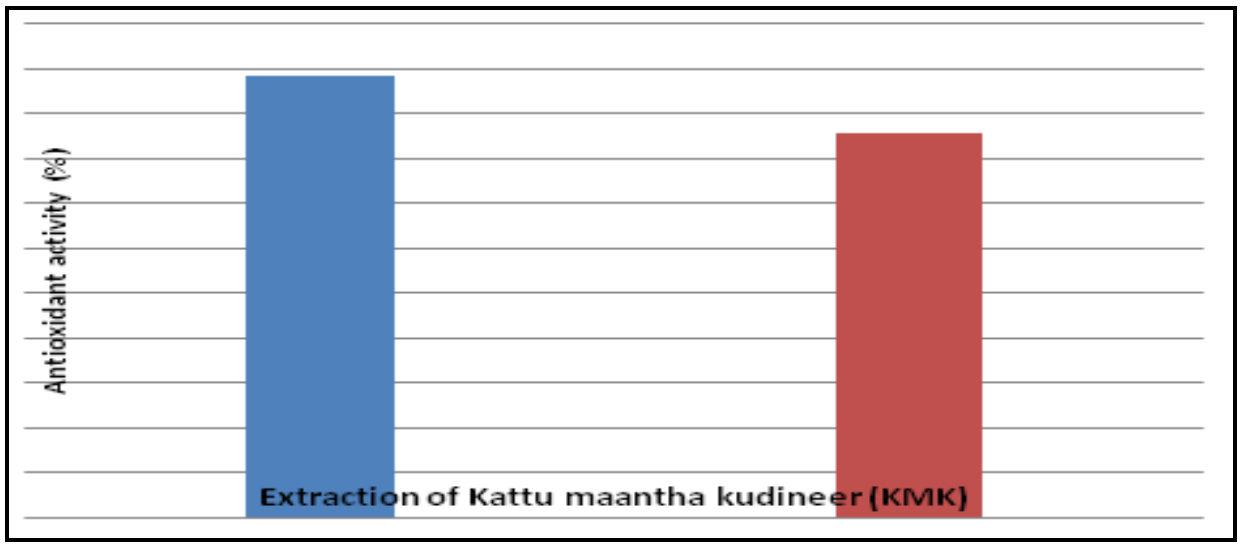
Quantitative analysis of free radical scavenging activity of sample **of *Kattu maantha Kudineer* (KMK)**

Time	0	5	10	15	20	25	30
KMK - OD	0.34	0.26	0.22	0.19	0.18	0.18	0.18
%	73.2	79.5	82.6	85.0	85.8	85.8	85.8
BHT - OD	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
Control - OD	1.27						

Determination of Total Phenolic Contents in *Kattu maantha Kudineer* (KMK)

S.No	Sample	Phenol mg (GAE)/g extract.
1	*Kattu maantha Kudineer* (KMK)	63.2

The total phenolic content of *Kattu Maantha Kudineer* was 63.2 mg gallic acid equivalents (GAE)/g extract.



Using DPPH free radical scavenging method, anti-oxidant activity of *Kattu Maantha Kudineer* was proven.

CONCLUSION

The results of the present study demonstrate that the drug *kattu maantha kudineer* has significant anti-oxidant activity using DPPH assay, contains zinc, calcium, iron, starch, reducing sugar. Toxic comnstituents like lead, mercury, arsenic were absent. In phytochemical analysis, ash value, pH, moisture content, crude fibre, tannin, saponin, flavonoids were estimated and total phenolic content of *kattu maantha kudineer* by *Folin* Ciocalteau colorimetric method was noted.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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