A COMPARATIVE PHYTOCHEMICAL STUDY OF DIFFERENT TYPES OF KAPIKACCHU SEEDS W.S.R. TO ITS USE IN PARKINSON’S DISEASE

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ABSTRACT

Ageing is a process of physical, psychological and social change in multi-dimensional aspects. Parkinson's disease is a slowly progressive disease of late adult life and is one of the most prevalent neurological disorders. It happens when nerve cell in the brain don’t produce enough of a brain chemical called dopamine. It is a disease of elderly and its prevalence increases from 1% in people over the age of 65 years to 5% in people over the age of 80 years and affects men and women equally. On the basis of signs and symptoms, Parkinson’s disease can be correlated with Kampavata a disease described under the heading of Vata Nanatmaja Vyadhi in Ayurveda. L-Dopa is precursor of the neurotransmitters dopamine. Many previous studies show that Kapikacchu (Mucuna pruriens (L.)DC.) is a rich source of L-Dopa. In market two types of seeds (black and white) are available and are being used simultaneously in the name of Kapikacchu. Phytochemical evaluation of black seeds of Kapikacchu has been done but phytochemical study of white seeds of Kapikacchu and their comparative phytochemical evaluation has not done yet. The aim of present article is to put forward the phytochemical profile of both types of Kapikacchu seeds and their comparative analysis also. Thus a conclusion can be reached as to which seeds (black or white) are more effective in treatment of Parkinson’s disease.

KEYWORDS: Parkinson’s disease, dopamine, Kampavata, Kapikacchu.
INTRODUCTION

Ageing is a process of physical, psychological and social change in multi-dimensional aspects. Parkinson’s disease is a progressive neurodegenerative disorder. It is the most common extra-pyramidal crippling disease affecting the older adults. It is a syndrome consisting of classical triad of resting tremor, bradykinesia and rigidity.\(^1\) The disease is increasing in its frequency with a worldwide incidence of 1-2 persons per 1000 population and becoming a major cause of disability in the aging society.\(^2\) This disease has remained a great problem in aging society which usually affects after the age of 50 years.

In Parkinson’s disease the basic pathologic changes is degeneration of a group of nerve cells deep within the centre of brain in an area called substantia nigra. These cells use Dopamine as their neurotransmitter to signal other nerve cells. As these cells degenerate & stop functioning, Dopamine fails to reach the areas of brain which leads to motor dysfunctions viz. resting tremor, bradykinesia and rigidity.

On the basis of signs and symptoms, Parkinson’s disease can be correlated with Kampavata a disease described under the heading Vata Nanatmaja disorders in Ayurveda. In time of Charaka and Sushruta cluster of symptoms like Kampa (tremor), Stambha (rigidity), Chestasanga (bradykinesia and akinesia), Vakvikriti (disturbance in speech) etc. were described in different contexts, and are clubbed as part of Vatika (Neurological) disorders.\(^3,4\)

As a separate clinical entity of Kampavata was first narrated by Acaraya Madhavakara (author of Ayurvedic treatise Madhava Nidana) under the name of “Vepathu”.\(^5\) It was the Basvarajiyam who for the first time gave a unanimous description by explaining the clinical picture of Kampavata and all clinical features described by him are similar to that of Parkinson’s disease. Symptoms of Kampavata such as karapadala kampa (tremors in hands &legs), Dehabhraman (postural instability), Matiksheen (Dementia) and Nidrabhagna\(^6\) (Insomnia) simulate to Parkinson’s disease.

Kapikacchu (Mucuna pruriens (L.)DC.) is a very well-known drug used in Ayurvedic classics. It is used as Balya, Vrishya, Brihankaraka and for Vata shaman.\(^7\) It is described as Vrishya Dravya in Samhita\(^8\) as well as Nighantu. Kapikachhu seeds contains L-Dopa which is indicated in Vatavyadhi and used as aphrodisiac.\(^6\) L-Dopa is precursor of the neurotransmitters Dopamine. In market two types of Kapikacchu seeds (black and white) are
available. An attempt has been made to evaluate comparative phytochemical analysis of black seeds and white seeds of Kapikacchu.

A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolic compounds, steroids, essential oils, lignins, resins and tannins etc.). They act as defense chemicals. Their absence does not cause bad effects in the plants.

Primary metabolites are essential to the growth of the cell. They are produced continuously during the growth phase and are involved in primary metabolic processes such as respiration and photosynthesis. Primary metabolites, which are identical in most organisms, include sugars, amino acids, tricarboxylic acids, the universal building blocks and energy sources. Secondary metabolites are the compounds which are derived by pathways from primary metabolic routes, and are not essential to sustain the life of cells. These compounds do not have a continuous production. Secondary metabolites are the end products of primary metabolites.

MATERIAL AND METHODS

Plant Sample
We had taken both types of Kapikacchu seeds (black and white seeds) from market by proper identification.

(a) Phytochemical screening
Freshly prepared extracts were tested for the presence of various active phyto compounds like phenols, tannins, flavonoids, proteins, carbohydrates, lipids, saponins, alkaloids and steroids.

Qualitative analysis of extracts to evaluate general phytochemical profile
The extracts obtained from the research drug were subject to qualitative examination as per the Pharmacopoeia of India (IP).

Tests for Carbohydrates\textsuperscript{[10]}
Molisch’s Test, Benedict’s test, Barfoed’s test, Fehling solution test.

Tests for Alkaloids\textsuperscript{[11]}
Mayer’s reagent test, Dragendorff’s reagent test, Hager’s Test.
Test for Amino acids
Ninhydrin test

Tests for Proteins\textsuperscript{[12]}
Biuret test, Xanthoprotic test, Millons test.

Test for saponins\textsuperscript{[13]}
Foam test

Test for glycosides\textsuperscript{[14]}
Borntrager’s test

Test for Phenolic Compound
The extract was taken in water and warmed to this. 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

Test for Flavonoids
Shinoda test

Test for Steroids\textsuperscript{[15]}
Salkowski reaction

Test for Tannins\textsuperscript{[16]}

(b) Thin layer Chromatography (T.L.C.)\textsuperscript{[17]}
Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Chromatography plates
T.L.C. plate coated with 0.25 mm layer of silica gel 60 F254 with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)
Activation of pre-coated Silica gel 60 F254
Plates were dried in hot oven at 1050 C for one and half hour.

Stationary phase
Thin layer chromatographic plates

Preparation of mobile solution:
Butanol: Acetic acid: Water = 8: 2: 2

Sample application
The respective extracts of samples were separately applied by a capillary tube to a thin layer chromatographic plate. The chromatogram was developed in the above solvent system, until the solvent front moved about three fourths of the length of the plate. The plate was then removed from the chamber, subsequently spray treated, air dried and examined under visual and short wave length ultraviolet light and the spots were recorded. This procedure was repeated five times for each extract of each sample and result documented as follows.

Visualization
Under Iodine Vapour.

Rf Value
Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

(c) UV Spectroscopy
Different chemicals were subjected for spectrometry in white light have specific affinity to absorb or to emit a particular range of wavelength. This wavelength is specific to that compound. Spectrometric analysis involves the measurement of the ability of the dissolved substance to absorb electromagnetic radiation of definite and narrow wavelength ranges. These absorptions are measured at wavelengths that are generally characteristic of the chemical composition of the dissolved absorbing substance. The UV visible spectra of the substance generally do not have a high degree of specificity but they are suitable for quantitative assays for many substances and useful as additional means of identification.
Calibration Curve of Standard L-dopa [18]
A stock solution of different concentration of L-dopa 41.62mg/ml, 39.4mg/ml, 59.6mg/ml and 52.5mg/ml was prepared in 0.1N formic acid in 100ml. Fluorescence intensity was measured for all the solutions at 630 nm emission wavelength.

Estimation of L-dopa in seeds of Kapikacchu
To determine the content of L-dopa in extract of each black and white seeds of kapikacchu (Mucuna pruriens L. DC.) sample, an accurately weighed 100 mg of dry aqueous extracts were transferred into 100 ml volumetric flask and 10 ml of 0.1N formic acid were added in each, sonicated for 10 min. and volume were made up to 100 ml with 0.1N formic acid. The extracts of each sample were filtered on a Whatman no. 1 filter paper, from which 1.0 ml of the each solutions were diluted to 1000 ml with 0.1N formic acid in volumetric flask. Finally solutions were diluted to get concentration of 204mg/ml, 198mg/ml, 226mg/ml and 215mg/ml and Fluorescence intensity were measured as above. The analysis was repeated for five times at different concentrations.

OBSERVATIONS AND RESULTS
Qualitative phytochemical analysis
Freshly prepared kapikachhu seeds extracts were tested for the presence of phyto constituents and the results are presented in Table 1.

Table no. 1: Qualitative phytochemical tests of extracts of kapikacchu Seeds
1. Carbohydrate test

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Name of test</th>
<th>Black seeds of Kapikacchu</th>
<th>White seeds of Kapikacchu</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Molisch test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>B.</td>
<td>Benedict test</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>C.</td>
<td>Barfoed’s test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>D.</td>
<td>Fehling test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

2. Alkaloids

| A. Dragondroff test | +ve | -ve | +ve | +ve |
| B. Wagner’s test    | +ve | -ve | +ve | -ve |
| C. Hager’s test     | +ve | +ve | +ve | +ve |
| D. Mayer’s test     | +ve | +ve | +ve | +ve |

3. Amino acids

| A. Ninhydrine test | +ve | +ve | +ve | +ve |
4. Proteins

A. Biuret test
-ve +ve -ve +ve
B. Xanthoproteic test
-ve +ve +ve +ve
C. Millon’s test
-ve +ve -ve +ve

5. Saponins

A. Foam test
-ve -ve +ve -ve

6. Glycosides

A. Borntragor’s test
+ve -ve -ve -ve

7. Phenolic compound

A. Phenolic test
+ve +ve +ve +ve

8. Steroids

A. Salkowski test
-ve -ve -ve -ve

9. Tannins

A. FeCl₃ test
+ve +ve +ve +ve
B. Lead acetate test
+ve +ve +ve +ve
C. Potassium dichromate test
+ve -ve +ve -ve

10. Flavonoids

A. Shinoda test
-ve -ve +ve +ve

Chromatography

Image showing the chromatography of Alcohol Extract

Table no. 2: Rf value of Kapikacchu sample

<table>
<thead>
<tr>
<th>Visualization under Iodine Vapour</th>
<th>Kapikacchu black seeds</th>
<th>Kapikacchu white seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf value</td>
<td>0.25, 0.30, 0.75</td>
<td>0.08, 0.25, 0.30, 0.39, 0.75</td>
</tr>
</tbody>
</table>
Ultra Violet Spectroscopy

(a) Kapikacchu white seeds powder

Table no. 3: comparison in concentration with absorbance on fixed wavelength

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wave length</th>
<th>ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>White seed 126 mg/ml</td>
<td>630</td>
<td>0.0125</td>
</tr>
<tr>
<td>White seed 156 mg/ml</td>
<td>630</td>
<td>0.0196</td>
</tr>
<tr>
<td>White seed 184 mg/ml</td>
<td>630</td>
<td>0.0245</td>
</tr>
<tr>
<td>White seed 198 mg/ml</td>
<td>630</td>
<td>0.0259</td>
</tr>
<tr>
<td>White seed 204 mg/ml</td>
<td>630</td>
<td>0.0269</td>
</tr>
<tr>
<td>White seed 226 mg/ml</td>
<td>630</td>
<td>0.0312</td>
</tr>
<tr>
<td>White seed 258 mg/ml</td>
<td>630</td>
<td>0.0489</td>
</tr>
<tr>
<td>L-dopa 41.62 mg/ml</td>
<td>630</td>
<td>0.0265</td>
</tr>
</tbody>
</table>

- 204mg/ml Kapikacchu white seeds powder solution shows 0.0269 Abs. which is equivalent to L-dopa 41.62mg/ml.
- Kapikacchu white seeds powder have aqueous extractive value- 20.36%. 1.001gm Kapikacchu white seeds powder have 0.204gm extract which is equivalent to L-dopa 0.04162gm.
- Kapikacchu white seeds powder have 4.157% L–Dopa concentration.

GRAPH NO.1: Comparison in concentration with absorbance on fixed wavelength

(b) Kapikacchu black seed powder

Table no. 4: Comparison in concentration with absorbance on fixed wavelength

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wave length</th>
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<tr>
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</tr>
<tr>
<td>Black seed 156 mg/ml</td>
<td>630</td>
<td>0.0196</td>
</tr>
</tbody>
</table>
226 mg/ml Kapikacchu black seeds powder solution shows 0.0312 Abs. which is equivalent to L-dopa 59.6 mg/ml.

Kapikacchu black seeds powder have aqueous extractive value 23.72%. 0.952 gm Kapikacchu black seeds powder have 0.226 gm extract which is equivalent to L-dopa 0.0596 gm.

Kapikacchu black seeds powder have 6.27% L-Dopa concentration.

**DISCUSSION**

**Phytochemicals**

- Fehling & Benedict test is done for to detect the presence of reducing sugar in a compound. Reducing sugar is any sugar that in a solution has an aldehyde and ketone group. Fehling test is positive in aqueous extract of black seeds which indicate presence of reducing sugar. Benedict test is positive in aqueous extract of white seeds of Kapikacchu which also indicate the presence of reducing sugar. Molisch test is positive in aqueous extract of each sample of seeds of Kapikacchu.

- Alkaloids are the naturally occurring compound that contain mostly basic nitrogen atom. These groups also include some related compound with natural and even weakly acidic
properties. Alkaloid’s test is positive in aqueous extract of each sample of seeds of Kapikacchu which indicate presence of alkaloids.

- Millon’s test is used to detect the presence of protein. This test is specific for testing the presence of tyrosine residues in protein. Tyrosine is an aromatic amino acid with a hydroxyl group. This test is positive in alcoholic extract of each sample of seeds of Kapikacchu which indicate the presence of protein.
- Ferric chloride test and Lead acetate test are used to detect the presence of Tannins. These tests are positive in both aqueous and alcoholic extract of each sample of Kapikacchu.
- Foam test is used to detect the presence of saponins. Foam test is positive in aqueous extract of white seeds of Kapikacchu.
- Salkowski test is used to detect the presence of steroids. This test is negative in each sample of seeds Kapikacchu.

**Thin layer chromatography**

TLC of alcoholic extract of seeds of Kapikacchu was developed in the mobile phase Butanol: Acetic acid: Water with 8: 2: 2 ratio, Stationary Phase Thin layer chromatographic plates (silica gel g) and Visualization in Iodine vapor.

**Rf value are as follows.**

- For the white seeds - 0.08, 0.25, 0.30, 0.39, 0.75.
- For the black seeds - 0.25, 0.30, 0.75

In Chromatography evaluation white seeds and black seeds have found some common RF value.

**Ultra Violet Spectroscopy**

According to observations Black Kapikacchu seeds have 6.27\% concentration of L-dopa which is greater than White Kapikacchu seeds (4.157\%).

**CONCLUSION**

In Qualitative analysis of extracts to evaluate general phytochemical profile like protein, amino acids, carbohydrates, alkaloids, tannins, phenolic compounds were present in each sample. Steroids were absent in each sample. Saponins were present in aqueous extract of white seeds. Glycosides were present in aqueous extract of black seeds. Flavonoids were present in aqueous and alcoholic extract of white seeds.
In UV analysis both types of seeds have L-DOPA concentration but black seeds have greater than white. So the black seeds of Kapikacchu seeds are better source of L-dopa. Thus on the basis of L-DOPA concentration it can be concluded that black seeds of Kapikacchu are more effective in treatment of Parkinson’s disease.

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