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Phytochemical Analysis of Selected Anti-Pyospermic Drug, Which Comprises of Natural Herbs

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Abstract

HA7 Choorna, composed of Strychnos potatorum, Curcuma longa, Coscinium fenestratum, Cassia fistula, Terminalia chebula and used for pyospermia in sub fertile male, was analyzed in vitro to find out its antioxidant capacity, phytochemical properties, availability of bioactive components and heavy metal contents. Pyospermia, the finding of more than 5 seminal pus cells/ high power field, has a negative relationship with seminal parameters hence the male fertility. Thus, the attention on pyospermia is important as an etiology of male subfertility. The HA7 Choorna was selected here due to its heavy usage against pyospermia in Ayurveda. In the assay, the components, tannins, flavonoids, terpenoids, phenol were found. Comparatively drug had a satisfactory antioxidant capacity. The heavy metals Pb, Cd, As, Hg were below the reference level. Two chemical compounds (Rf = 0.75 and 0.76) were found under the Chromatography/TLC. Thus, the drug has natured a good antioxidant level which can fight against the bad effect of free radicle synthesized in pyospermia due to the finding of phytochemicals in it. The drug is safe from mentioned heavy metals. Finally, upon the result the other studies such as microbiological, phytochemical, toxicological studies should be carried out on drug to ascertain its safety, efficacy.

Key Words: male infertility; pyospermia, anti-pyospermic drug, phytochemicals, antioxidant, metals

Introduction

Male factor infertility, which accounts 30-40% of the infertility (1) has become a psychosocial issue in the modern society. The men's fertility basically depends on the quality of seminal parameters such as seminal count, volume, motility and morphology. The deviation of these parameters from their normal reference range could lead to male infertility. Amidst the conditions which can cause poor quality semen, pyospermia (presence of more than 1 million leukocytes in 1 ml semen or more than 5 pus cell/HPF of microscope) (2), (3) is predominant. The pyospermia situation could compromise male fertility, as it increases the level of oxidative stress, proteolytic enzymes which badly effect on the sperm

quality (2). Thus, the attention of treatment and prevention of pyospermia is much important. In country like Sri Lanka which practice the Indigenous medicine, the pyospermia is treated with a preparation (HA7 Choorna) which is a blend of natural herbal flora. The community in the developing as well as developed countries has been using the traditional herbal medicines for a thousand of years for curing the ailments in owing to its natural origin and lesser side effects in comparison to the synthetic drugs (4). In olden days, the traditional practitioners used to treat patients on an individual basis and prepared the drug in small amount according to the requirement of the patient. However, the situation has changed currently to manufacture the herbal medicines on a large scale, so as the manufacturers come across many problems such as unavailability of good quality raw material, authentication of raw material, unavailability of standards and proper standardization methodologies, parameters of quality control. Hence, the quality control and standardization of herbal drug products is very important for the in order to minimize the adverse effects and to maintain batch to batch consistency. Otherwise the remedy could itself become an etiology of a disease (5). WHO (World Health Organization), in 1992 has issued comprehensive guidelines for the quality control and standardization of herbal drugs and these guidelines are very useful for ensuring quality, safety and therapeutic efficacy, batch to batch consistency? Thus, in the present study the anti-pyospermic Ayurveda drug HA7 Chooorna was scientifically analyzed.

Methods and Methodology

Preparation of the drug:

The HA7 Choorna was prepared by correct identification and authentication of the plant materials followed by washing, drying under shade, powdering and mixing the barks of them respectively and using standard protocols (Ayurveda Saara Sangrahaya) under the supervision of an Ayurveda medical practitioner. The final product of HA7 Choorna was found as a yellow colored powder with the characteristic Turmeric smell.

Phytochemical analysis of HA7 Choorna (6):

The drug powder (HA7 Choorna) in aqueous as well as methanolic solutions were assessed for qualitative analysis of phytochemicals.

Tannin: The drug powder (0.3 g) was boiled with water and methanol in 2 test tubes separately and filtered. The 5.0 ml of each filtrate was treated with 3 drops of 0.1% of ferric chloride to observe the brownish green or blue-black colorization for the presence of tannin.

Tests for Flavonoids (Shinoda Test): Either solution of the drug powder (2.0 ml) were mixed with a magnesium piece and added a 4 drops of Conc. HCl to observe the formation of pink color for the presence of flavonoids.

Test for Terpenoids: Each solution of the drug powder (5.0 ml) were mixed with 2.0 ml of chloroform and added 3.0 ml of Conc. H_2SO_4 to observe the formation of red brown color for terpenoids.

Test for phenol: Each solution of the drug powder (2.0 ml) were mixed with 2 drops of 2% of $FeCl_3$ to observe the formation of violet green color for phenol.

Tests for Steroidal Glycosides (Liebermann's Test): Each solution of the drug powder (2.0 ml) were mixed with 2.0 ml of acetic acid and 2.0 ml of chloroform. The mixtures were cooled and added with Conc. H_2SO_4 to observe the formation of green blue color for steroidal glycosides.

Tests for Cardiac Glycosides (Keller-Kiliani Test): Each solution of the drug powder (5.0 ml) were mixed with glacial acetic acid (2.0 ml) and a drop of 2.0% of $FeCl_3$. Eventually, a 1.0 ml of volume of Conc. H_2SO_4 was added to observe the formation of brown ring which indicate Cardiac Glycosides.

Test for coumarin: Each solution of the drug powder (2.0 ml) were mixed with 3 drops of 1% KOH solution to observe the formation of yellow color for coumarin.

Test for Saponins: The drug powder, around 0.5 g was placed in a test tube and mixed vigorously with 5.0 ml of distilled water to observe a stable foam.

Determination of total antioxidant level of HA7 Choorna (7):

Fresh FRAP reagent was prepared by mixing the 25.0 mL of acetate buffer (300 mM; pH 3.6), 2.5 mL of TPTZ solution (10 mM) and 2.5 mL of ferric chloride solution (20 mM) and incubating them for 15 min at 37 °C. HA7 Choorna (1.0 g) was extracted in 10 ml of distilled water and methanol. FRAP solution (2.85 ml) was mixed well with 159.00 µl of each extract of Choorna sample. The absorbance of the mixtures was measured at 593 λ, against the blank (a mixture of FRAP reagent and distilled

water/methanol) at 0 min and 4 min (after keeping at 37 °C).

The ascorbic acid (1000 µg/ml) was considered as the standard.

The equation for FRAP value was as follows.

FRAP value = absorbance of test sample at (0 min - 4 min) x 2

absorbance of standard sample at (0 min - 4 min)

In the equation, 2 is the FRAP value of the standard

Thin layer chromatography (TLC fingerprint):

Preparation of sample: Soxhlet extractor was used to extract the Choorna (8.0 g) in to 150.0 ml of methanol and the methanol in the concentrated extraction was evaporated with the rotary evaporator.

Thin layer chromatography (TLC): Two separate mobile phases were used, and fingerprint was visualized with iodine vapor, vanillin sulphate and UV light.

Mobile phase 1/Solvent 1 = 6:4:1:1.5 Cyclohexane: Dichloromethane: Ethylacetate: Methanol

Mobile phase 2/Solvent 2 = 1:1:0.3 Dichloromethane: Cyclohexane: Methanol

Heavy metal analysis of HA7 Choorna:

Wet digestion of the sample: Choorna (0.50 g) was boiled with Conc. HNO3 (5 ml) in a water bath for 60 min until it became to a clear solution. Then the solution was made in to a clear solution by heating with hydrogen peroxide. Finally, the solution was neutralized and was tested for heavy metals (Hg, As, Pb, Cd) with atomic absorption spectrophotometer (AOAC method).

Result and Discussion

Phytochemical analysis:

Table 1: Phytochemical screening of the HA7 Choorna

Chemical compounds	Aqueous extract	Methanolic extract
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	+
Phenol	+	+
Steroid glycosides	-	-
Cardiac glycosides	-	-
Coumarins	-	-
Saponins	-	-

present +, absent -

As seen in Table 1, the preliminary phytochemical screening of aqueous and ethanolic extracts of the Choorna indicated the presence of Tanins, Flavonoids, Terphenoids, and Phenols. It did not indicate the presence of Coumarins, Saponins and Glycosides.

Terpenoids

Terpenoids is an aromatic compound. It is also known as isoprene. Terpenoids, found to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties. In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties (8)

Phenols

The phenol reduces inflammation via preventing the production of oxygen free-radicals by leucocytes (9). Further phenol exhibits biological effect such as, inhibition of carcinogenesis, host-mediated antitumor activity, antiviral activity, inhibition of active oxygen, such as inhibition of lipid peroxidation and lipoxygenase, xanthine oxidase, and monoamine oxidase. (10)

Flavonoids

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure (11). Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities.

Tanins

The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The tannin has shown antimicrobial activities as well. The growth of many yeasts, fungi, virus and bacteria was inhibited by tannins. Among the other physiological effects of tanins, increasing the blood clotting, reducing the blood pressure, decreasing the serum lipid level and modulating immune responses are prominent (12). Antioxidant, anti-inflammatory and antimicrobial activities of Tanins, flavonoids, terpenoid and phenols, present in the Choorna may be responsible for its healing property pyospermia.

Determination of total antioxidant level:

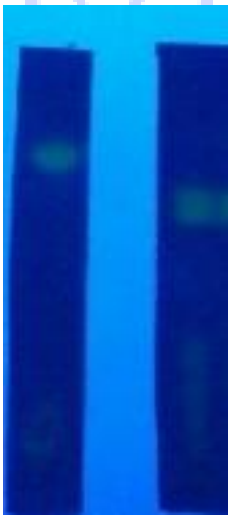
Table 2: Determination of FRAP value for HA7 Choorna

Standard Concentration of vitamin C (µg/ml)	FRAP Value for methanol extract	FRAP Value for aqueous extract
1000	6.8	8.8

Total antioxidant level of the Choorna in methanol and aqueous extract were calculated for each concentration considering the antioxidant value of vitamin C as 2. Hence, the total average antioxidant capacity of the Choorna was 6.8 and 8.8 µg/ml, for the methanol and aqueous extract respectively (Table 2). The antioxidant capacity of the current drug (Choorna) could be due to phytochemicals such as tannin, flavonoid, terphenoid and phenol, present in the Choorna. The tannin is considered as a superior antioxidant. Hence, the Choorna is considered to possess remarkable antioxidant effect against the oxidation. The pus cells are considered as the sources of reactive oxygen species. This high antioxidant property of the drug could be a one reason for the effect of the HA7 Choorna for pyospermia.

TLC fingerprint:

TLC of Soxhlet methanol extract



A B

Figure 1: UV visualization (265 λ) of TLC for Soxhlet methanol extract of HA7 Choorna
(A – Mobile phase1/ solvent 1, B – Mobile phase2/ solvent 2)

One visible spot was seen in the TLC fingerprint of solvent 1 (Figure 1)
The Rf value = 5/6.5 = 0.76
One visible spot was seen in the TLC fingerprint of solvent 2 (figure1)
The Rf value = 4.5/6 = 0.75
Both solvent mixtures were able to separate two component swith the Rf value of 0.76 and 0.75.

Heavy metals in the HA7 Choorna:

Table 3: Concentration of heavy metals in HA7 Choorna

Heavy metal	Concentration (µg/g)
As (Arsenic)	0.30 ± 0.11
Cd (Cadmium)	0.04 ± 0.01
Pb (Lead)	0.003 ± 0.001
Hg (Mercury)	0. 00 ± 0.00

Heavy metals are commonly defined as those having a specific density of more than 5 g/cm3. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. Heavy metals have been used by human being for various purposes for thousands of years. Inclusion of heavy metals to herbal drugs could occur via the raw material or due to contamination occur during the processing, storage or transportation (13). The side effect of the heavy metal ingestion depends on the concentration as well as the time period of exposure to heavy metal. Since the Choorna is ingested by the patients for a longtime duration (minimum 3 months) the detection of basic heavy metals was an obligation in the analysis of HA7 Choorna for the quality. As the metals are absorbed by the plant from soil, a certain amount of heavy metal could be possible to find in an herbal drug. The permissible limits of the heavy metals (13) in an herbal product as per WHO (World Health Organization) and FDA (Federal Drug Administration) is shown in the Table 4.

Table 4: The WHO permissible limit of heavy metals in an herbal preparation

Heavy metal	Concentration
As (Arsenic)	10^3 ng/g
Cd (Cadmium)	0.3 µg/g
Pb (Lead)	10 µg/g
Hg (Mercury)	1.0 µg/g

Heavy metal analysis of Choorna (Table 3) revealed, the concentration of As, Cd, Pb and Hg presence in the sample is well below the WHO permissible limits. Hence the drug is safe to use. The similar toxic study has been carried out with Yakrit Plihantak Churna and found to have no toxic materials as it was seen in the current Choorna (14).

Conclusion

According to the results the drug contained a good antioxidant capacity which can fight the harm full effect of free radicles synthesized by the pyospermia. This could be due to the various phytochemicals available in it such as Tannin, Terphenoid, Flavonoids and phenol. Further the drug is safer from heavy metals such as Pd, Cd, Hg and As. The drug contains 2 active chemical compounds (Rf =0.75,0.76) which dissolve in methanol. Finally, the studies such as microbiological, phytochemical studies as well as toxic studies etc. should also be carried out on the drug to ascertain its safety, efficacy by making this study a platform.

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