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A PHYTOCHEMICAL ANALYSIS & ANTIMICROBIAL STUDY OF SINAPIS ALBA-A RESEARCH STUDY

Dr. Vinod Ranga*[1] and Dr. Sneha Ranga[2]

[1]Principal & Professor Dept. of Dravyaguna,

[2]Professor Dept. of Rachana Sharir,

Mai Bhago Ayurvedic Medical College, Ferozepur Road, Shri Muktsar Sahib- Punjab.

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***Corresponding Author**
Dr. Vinod Ranga
Principal & Professor Dept. of Dravyaguna, Mai Bhago Ayurvedic Medical College, Ferozepur Road, Shri Muktsar Sahib- Punjab.

ABSTRACT

Charak Samhita is the oldest grantha of Ayurveda. It was written by agnivesha in 1000 B.C. Acharya Charaka refined it in 2nd Century B.C. Later on some more topics are added by Dridhabala in it in 4th A.D. Sinapis alba has been described in Kandughna Mahakashaya in Charaka Samhita. It has also described among Asthapanopaga and Shirovirechanopaga Dravya. Charaka has given various Synonyms of Sinapis alba like Gaur Sharshap, Rakshoghni and Siddharthak. The drug has been used in various diseases in Charaka Samhita. In this study aqueous extract has been found more effective than other extracts. Ayurveda had preferred Sinapis alba with water in many diseases. Kwatha is equal to water extract, and it is proved that the water soluble contains are more beneficial for human body, therefore the aqueous extract of Sinapis alba has been found more efficacious.

KEYWORDS: Charak Samhita, Sinapis alba, Dravya, Sharshap, Kwatha.

INTRODUCTION

The Indian sages thousands of years ago enscripted their knowledge about life and the fundamentals of Ayurveda in the oldest scriptures known to mankind, namely the Rig Veda, Yajur Veda, Athrva Veda and Sam Veda. Ayurveda is regarded as the Upaveda or sub-scripture of the Atharva Veda. This Veda contains abundance of information on how to keep oneself healthy and to combat diseases. There are a lot of diseases which are caused by microbes. Microbes are also described in Ayurvedic literatures under the heading Krimi. In Charak Samhita the description of krimis and different krimi rogas is found in detail. The

other Acharya like Bhel, Sushruta, Harita, Vagbhata had also depicted sufficiently regarding the pathogenic krimis. Charaka has clubbed the group of herbs useful in the treatment of krimi under one heading called "Krimighna mahakashaya". A special group of plants named "Rakshoghna gana" is also described as group of plants possessing krimighna properties in many Ayurveda literatures. These plants are proving their self very effective against the microbes. Among these plants one is Shveta Sarshap which is described chiefly in grahbadha, Amanushopratishedha and as a dhoopan dravya in various places in Charaka Samhita, Sushruta Samhita and many Nighantu. That's why the drug chosen for the Antimicrobial Study was Sinapis alba.

AIMS AND OBJECTIVE OF THE STUDY

1-To analyze the Phytochemical in order to determine the different active constituents of Sinapis alba.

2-Antimicrobial activity evaluatation by culture and sensitivity test.

3-Assessment of the drug on Ayurvedic parameters as described in Dravyaguna (Namrupa Vigyana).

MATERIALS AND METHODS

This study is divided into various parts. Each part has its importance and has done very carefully to avoid any kind of mistake.

A. Collection and identification of plant material

Fresh seeds of Sinapis alba were collected randomly from the semi-arid region of Narnaul, Haryana, INDIA. The plants and the parts screened, together with their families and vernacular names. The taxonomic identities of these plants were confirmed by Dr. Naresh Khemani, Associate Professor, Department of Dravyaguna, N.I.A., Jaipur. Fresh seeds were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

B. Physiochemical parameters

1. Organoleptic features
2. Foreign matter
3. Loss on drying
4. Ash Value

I. Total ash

II. Acid insoluble ash

III. Water soluble ash

5. Extraction in different solvents

C. Phytochemical Analysis

1. Detection of Alkaloids
2. Detection of Glycosides
3. Detection of Tannins
4. Detection of Saponins
5. Detection of Carbohydrates
6. Detection of Starch
7. Detection of Phenols
8. T.L.C.

D. Qualitative Examination

1. Calcium
2. Iron
3. Magnesium
4. Manganese
5. Phosphorus
6. Potassium
7. Sulphur

Equipments required

1. Weighing machine (Digital balance)
2. Silica Crucible
3. Gooch crucible
4. Whatman's filter paper
5. Beaker, Conical flask and Test tubes.
6. Measuring cylinders
7. Muffle furnace
8. All the chemical as required

DRUG REVIEW

Regional Name	–	Varnagular name
Sanskrit	–	Gaur Sarshap
Hindi	–	Safed Sarson
Bangali	–	Shwet Sarshe
Marathi	–	Shwet Siras
Gujrati	-	Sharshav
Punjabi	–	Sareyan
Kannad	–	Biliya sasev, Sasve
Telgu	–	Aavalu
Tamil	–	Vasamby
Malyalam	–	Shirshi
Arabic	–	Hurf, Urfe abiyad
Pers	–	Sarsaph
Eng	-	White mustard
Latin	-	Sinapis alba Linn.

Rasa Panchaka

Rasa panchaka are the five fundamental principles of Ayurveda on which the whole drug action depends. We can say that the pharmacodynamics and pharmacokinetics of a drug depends on its rasa panchaka. The rasa panchaka of the drug Sinapis alba are:

SR. NO.	PANCHAKA	DESCRIPTION
1.	Rasa	Katu, Tikta
2.	Guna	Snigdha, Tikshna, Ushna
3.	Veerya	Ushna
4.	Vipaka	Katu
5.	Prabhava	Kandughna

Properties and actions

Karma: Kandughna, Varnya, Kushthaghna, Lekhana, Jantughna, Vednasthapana, Snehana, Dipana, Krimighna, Plihanashana, Hrdayottejaka.

Prayoga

Twakavikara – Kandu - Vicharchika – Dadru

Vrana – Visphota – Apachi

Udarda – Sheetapitta

Kustha

Vatarakta

Vatvyadhi – Shoola – Shotha – Urustambha

Shlipada

Krimiroga

Udarvikara – Shoola – Kaphodara

Plihavriddhi

Agnimandhya – Gulma

Mutraghata

Rajorodha

Daurbalya

Officinal Part: Seeds, seed oil and leaves.

Therapeutic dose

Seed powder - 2-4 g & Seed oil – 2-4 drops

Route of administration

Oral, Nasal, Local.

MICROBIOLOGY

Scientific classification

- Kingdom - Bacteria
- Phylum - Firmicutes
- Class - Bacilli
- Order - Lacto bacillales
- Family - Streptococcaceae
- Genus - S. Pyogenes
- Binomial Name - Streptococcus pyogenes

By Rasenbach (1884)

ANTIMICROBIAL STUDY

Preparation of Solvent Extracts

1-Preparation of Methanol Extract

The air dried powdered 50 g (accurately weighed in an electronic balance) sample of Sinapis

alba was taken in a conical flask (of Quantity 250ml) containing 100 ml methanol and plugged with cotton wool. It was then kept in a rotary shaker machine under the parameters of 38°C temperature and 200-220 rounds per minutes of shaking for the time period of 12 hours.

After complete shaking, the flask was removed from the shaker machine and the solvent was kept untouched for about 3 hours. After that the solvent was filtered through Whatmans no. 1 filter paper. The filtrate was put in a pre weighed sterile glass Petri dish and then kept in an oven under the temperature of 60°C till the complete evaporation of the solvent. The temperature should not increase than 60°C as the boiling point of methanol is 60°C and if the temperature of the solvent increases than the boiling point of methanol, the extract can be debilitated. The extract was cooled in desiccators for 30 minutes, weighed without delay and kept in eppendorf tube & covered with a moisture free zip seal plastic cover.

It was observed that extract obtained was oily & black in colour.

2-Preparation of Ethanol Extract

The air dried powdered 50 g (accurately weighed in an electronic balance) sample of Sinapis alba was taken in a conical flask (of Quantity 250ml) containing 100 ml ethanol and plugged with cotton wool. It was then kept in a rotary shaker machine under the parameters of 38°C temperature and 200-220 rounds per minutes of shaking for the time period of 12 hours.

After complete shaking, the flask was removed from the shaker machine and the solvent was kept untouched for about 3 hours. After that the solvent was filtered through Whatmans no. 1 filter paper. The filtrate was put in a pre weighed sterile glass Petri dish and then kept in an oven under the temperature of 90°C till the complete evaporation of the solvent. The temperature should not increase than 90°C as the boiling point of ethanol is 90°C and if the temperature of the solvent increases than the boiling point of ethanol, the extract can be debilitated. The extract was cooled in desiccators for 30 minutes, weighed without delay and kept in eppendorf tube & covered with a moisture free zip seal plastic cover.

It was observed that extract obtained was oily & black in colour.

3-Preparation of Aqueous Extract

The air dried powdered 50 g (accurately weighed in an electronic balance) sample of Sinapis alba was taken in a conical flask (of Quantity 250ml) containing 100 ml distilled water and

plugged with cotton wool. It was then kept in a rotary shaker machine under the parameters of 38°C temperature and 200-220 rounds per minutes of shaking for the time period of 12 hours.

After complete shaking, the flask was removed from the shaker machine and the solvent was kept untouched for about 3 hours. After that the solvent was filtered through Whatmans no. 1 filter paper. The filtrate was put in a pre weighed sterile glass Petri dish and then kept in an oven under the temperature of 100°C till the complete evaporation of the solvent. The temperature should not increase than 100°C as the boiling point of distilled water is 100°C and if the temperature of the solvent increases than the boiling point of distilled water, the extract can be debilitated. The extract was cooled in desiccators for 30 minutes, weighed without delay and kept in eppendorf tube & covered with a moisture free zip seal plastic cover.

It was observed that extract obtained was oily and dark brown in colour.

4-Preparation of Benzene Extract

The air dried powdered 50 g (accurately weighed in an electronic balance) sample of Sinapis alba was taken in a conical flask (of Quantity 250ml) containing 100 ml benzene and plugged with cotton wool. It was then kept in a rotary shaker machine under the parameters of 38°C temperature and 200-220 rounds per minutes of shaking for the time period of 12 hours.

After complete shaking, the flask was removed from the shaker machine and the solvent was kept untouched for about 3 hours. After that the solvent was filtered through Whatmans no. 1 filter paper. The filtrate was put in a pre weighed sterile glass Petri dish and then kept in an oven under the temperature of 90°C till the complete evaporation of the solvent. The temperature should not increase than 90°C as the boiling point of benzene is 90°C and if the temperature of the solvent increases than the boiling point of benzene, the extract can be debilitated. The extract was cooled in desiccators for 30 minutes, weighed without delay and kept in eppendorf tube & covered with a moisture free zip seal plastic cover.

It was observed that extract obtained was oily and yellow in colour.

5-Preparation of Petroleum Ether Extract

The air dried powdered 50 g (accurately weighed in an electronic balance) sample of Sinapis alba was taken in a conical flask (of Quantity 250ml) containing 100 ml petroleum ether and

plugged with cotton wool. It was then kept in a rotary shaker machine under the parameters of 38°C temperature and 200-220 rounds per minutes of shaking for the time period of 12 hours.

After complete shaking, the flask was removed from the shaker machine and the solvent was kept untouched for about 3 hours. After that the solvent was filtered through Whatmans no. 1 filter paper. The filtrate was put in a pre weighed sterile glass Petri dish and then kept in an oven under the temperature of 90°C till the complete evaporation of the solvent. The temperature should not increase than 90°C as the boiling point of petroleum ether is 90°C and if the temperature of the solvent increases than the boiling point of petroleum ether, the extract can be debilitated. The extract was cooled in desiccators for 30 minutes, weighed without delay and kept in eppendorf tube & covered with a moisture free zip seal plastic cover.

It was observed that extract obtained was oily and yellow in colour.

An overview of extracts in different solvents

S. No.	Solvent used	Extract state	Extract colour
1.	Methanol	Oily	Black
2.	Ethanol	Oily	Black
3.	Aqueous	Oily	Dark brown
4.	Benzene	Oily	Yellow
5.	Petroleum Ether	Oily	Yellow

OBSERVATIONS

The zone of inhibition was observed in all the media plates against all the five pathogenic bacterial strains which were taken for the sensitivity study.

RESULTS

The final result is that the antimicrobial activity of water extract of Sinapis alba in concentration of 30 mg & 50 mg was found very highly sensitive against Pseudomonas aeruginosa (MTCC No. 424). The concentration of 15 mg of the extract was also found highly sensitive against Pseudomonas aeruginosa (MTCC No. 424) along with all the three concentrations against Staphylococcus aureus (MTCC No. 3160) and Salmonella typhi (MTCC No. 733) and the drug was moderate sensitive against the Streptococcus pyogenes (MTCC No. 1928) and Escherichia coli (MTCC No. 901) in all the concentrations. The antimicrobial activity of methanol extract of Sinapis alba in concentration of 50 mg was

found moderate sensitive against Staphylococcus aureus (MTCC No. 3160). The result of antimicrobial activity of ethanol extract of Sinapis alba was found less sensitive against all the bacteria in all the three concentrations.

Table showing sensitivity result (Inhibition Zone in m.m.) of five different extract of Sinapis alba in 3 concentrations against the five microbial cultures.

Sr. No.	Extract	Conc.	1928 Strepto. Pyogenes	3160 Staphy. Aureus	901 E. Coli	424 Pseudo. Aeruginosa	733 Sal. typhi
1.	Methanol	15g	I.Z. = 6	I.Z. = 8	I.Z. = 7	I.Z. = 6	I.Z. = 7
		30g	I.Z. = 6	I.Z. = 8	I.Z. = 8	I.Z. = 7	I.Z. = 8
		50g	I.Z. = 7	I.Z. = 9	I.Z. = 8	I.Z. = 7	I.Z. = 8
2.	Ethanol	15g	I.Z. = 6	I.Z. = 6	I.Z. = 6	I.Z. = 6	I.Z. = 7
		30g	I.Z. = 7	I.Z. = 6	I.Z. = 7	I.Z. = 6	I.Z. = 8
		50g	I.Z. = 7	I.Z. = 7	I.Z. = 7	I.Z. = 7	I.Z. = 8
3.	Petro. Ether	15g	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4
		30g	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4
		50g	I.Z. = 5	I.Z. = 5	I.Z. = 5	I.Z. = 5	I.Z. = 5
4.	Benzene	15g	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4
		30g	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4	I.Z. = 4
		50g	I.Z. = 5	I.Z. = 5	I.Z. = 5	I.Z. = 5	I.Z. = 5
5.	Water	15g	I.Z. = 9	I.Z. = 12	I.Z. =10	I.Z. = 14	I.Z. = 12
		30g	I.Z. = 9	I.Z. = 13	I.Z. =10	I.Z. = 15	I.Z. = 13
		50g	I.Z. = 9	I.Z. = 13	I.Z. =10	I.Z. = 15	I.Z. = 13

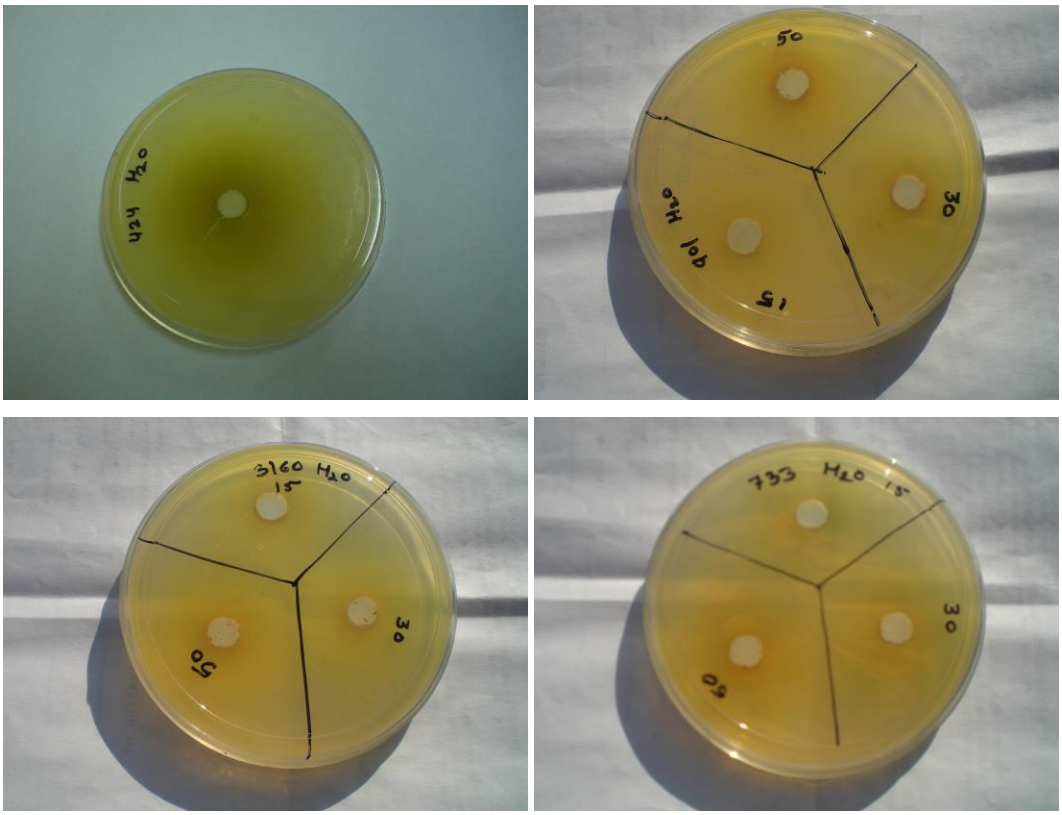
DISCUSSION

In this study aqueous extract has been found more effective than other extracts. Ayurveda had preferred Sinapis alba with water in many diseases. Kwatha is equal to water extract, and it is proved that the water soluble contains are more beneficial for human body, therefore the aqueous extract of Sinapis alba has been found more efficacious. The Aqueous ext. of Sinapis alba has exhibited high sensitivity against Staphylococcus aureus. Sinapis alba acts on most of the diseases caused by Pseudomonas aeruginosa like Krimiroga, Udarvikara – Shoola – Kaphodara, Plihavriddhi, Agnimandhya – Gulma, Mutraghata, Rajorodha, Daurbalya, Bhoot-grahabadha, Dantavikara, Karnavikara, Apsmara, Visha– Keetadansha and Netravikara, Twakavikara – Kandu - Vicharchika – Dadru, Vrana – Visphota – Apachi, Udarda – Sheetapitta, Kustha, Vatarakta, Vatvyadhi – Shoola – Shotha – Urustambha, The Aqueous ext. of Sinapis alba has exhibited high sensitivity against salmonella. Salmonella typhi is the causative factor for the enteric fever and food poisoning. In Ayurveda, Sinapis alba has been

prescribed as dhupana dravya at many places. The Dhupana is indicated for sashtragara, Sutikagara etc places. Dhupana is similar to modern fumigation of operation theaters and hospitals. Sinapis alba is also indicated for vrana dhupana, kasa, and skin infections in Ayurveda. These correlation supports to the results of aqueous extract against staphylococcus aureus.

The Aqueous ext. of Sinapis alba has exhibited moderate sensitivity against E.coli, E.coli is the main causative organism for gastrointestinal infection like peritonitis and diarrhea etc. Sinapis alba is indicated in Atisara (diarrhoea) and udara roga (gastro intestinal diseases) at many places in Brihattrai.

Antimicrobial result of Aqueous Extract of Sinapis alba



MTCC No. 3160 - Staphylococcus aureus
MTCC No. 901 - Escherichia coli
MTCC No. 424 - Pseudomonas aeruginosa
MTCC No. 733 - Salmonella typhi

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