

Screening of Antibacterial and Antifungal Activity of Gandhaka Rasayana- an Ayurvedic Formulation

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Research Article

Abstract: Introduction: Antimicrobial agents are commonly used nowadays for treating various bacterial and fungal infections. But still most of the physicians are unable to treat these infections appropriately due to hindrances like development of resistance, adverse effects, patient affordability etc. Gandhaka Rasayana formulation mentioned in Ayurvedic Texts can be an ideal replacement for treating various infectious diseases. Assessment of its antibacterial and antifungal activity may provide scientific evidence for the study. **Method:** Gandhaka Rasayana prepared with classical reference was subjected for analysis and invitro antibacterial and antifungal activity by cup plate method. Different concentrations of the drug was tested against bacteria like Staphylococcus aureus, Pseudomonas auregenosa and E coli and fungi Aspergillus niger, Cryptococcus neoformans, Candida albicans and Trycophytum rubrum taking Fluconazole as standard for comparison. **Results:** Gandhaka Rasayana solutions in different concentrations showed a significant zone of inhibition against three strains of bacteria (22-30mm) and four strains of fungi (16-25mm) when compared to Fluconazole (22mm) and control. As Gandhaka Rasayana contains Fe (433.84), Cu (32.77), Ni (10.32) Zn (10.32), Ca (0.84%), Na (0.17%), As (12.14), triclinc cells, carbohydrates, steroids and tannins it may contribute to promote positive health and vigour by increasing the immunity, thus making the body resistant against disease causing factors. **Conclusion:** Gandhaka Rasayana showed a significant antibacterial and antifungal activity, but antibacterial activity was significant compared to antifungal activity.

Key words: Gandhaka Rasayana, Antimicrobial agents, Fluconazole.

Introduction

Amongst the massive world of medicines, antimicrobial agents are one of the widely used and misused drugs available to medical profession. Once upon a time they were called as the magic bullets for their great contribution in curing an infection, but recently these bullets also backfire in way of causing resistance, decreasing their effectiveness and causing side effects.¹ In recent years number of antimicrobial agents have been discovered, but unfortunately even with the advent of

numerous AMA, still most of the physicians are unable to treat these infection appropriately due to hindrances while treating these infection like (a) development of resistance (b) adverse effects and (c) patient affordability etc. Though the centre for diseases control and prevention has taken steps to curb antimicrobial resistance, still it is flaring at a rampant rate due to over prescribing of these drugs. This over prescribing is driven largely by patient demand, time pressure on clinicians and diagnostic uncertainty, fuelling an ever-increasing need for newer drugs.² Making antibacterial drug therapy effective safe and affordable has been the focus of interest during recent years.³ Some of the Ayurvedic formulations may be considered as an ideal replacement for treating certain bacterial and fungal infection. Gandhaka Rasayana is one such drug mentioned in Ayurvedic text having a wide range of therapeutic properties as well as prophylactic activity. It is considered as a Rasayana (rejuvenator) which promotes positive health and vigor, retards aging process and increases longevity in individuals by increasing immunity in the body, thus making the body to resist against the factors causing ailments. It is extensively used by various physicians in different diseases like Kustha(skin disorders), Kasa(cough), Shwasa(asthama), Kshaya(Tuberculosis) etc.⁴ Antimicrobial agents usually act against microorganisms only without having any additional therapeutic properties i.e. promoting positive health and increasing the immunity like Gandhaka Rasayana. With the advent of new drug delivery systems, advancement in drug technology sciences, strict FDA guidelines for drug approval, growing health awareness in mass, it is desirable to study the principles and practice of Rasashastra by utilizing the facilities of modern medical science. Hence to provide statistical validation and data this study was undertaken.

Pharmaceutical preparation

Gandhaka Rasayana was prepared according to the reference of Yogaratnakar Rasayanadhikara⁴ by subjecting Shodhita Gandhaka¹⁴ (purified Sulphur acc to Ayurveda) to 8 bhavanas (trituration) of cow's milk, followed by 8 bhavanas of Chaturjataka kwath (*Cinnamomum zeylanicum*, *Elettaria cardamomum*, *Mesua ferrea*, *Cinnamomum tamala* decoction), Guduchi swarasa (*Tinospora cordifolia* juice), Haritaki kwath (*Terminalia chebula* decoction), Bibhitaki kwath (*Terminalia belerica* decoction), Amalaki swarasa (*Embllica officinalis* juice) Shunti kwath (dry *Zingiber officinale* decoction), Bringaraj swarasa (*Eclipta alba* juice) and Ardraka swarasa (*Zingiber officinale* juice). After completion of bhavana the product was mixed with equal quantity of powdered sugar. Total of 72 bhavanas were given to shodhita Gandhaka. After 8 bhavanas of each drava dravya (specified liquid) the bhavita Gandhaka was dried completely before starting the next bhavana. After 72 bhavanas it was dried completely. To this equal quantity of powdered sugar was added.

Analytical study

Gandhaka Rasayana was subjected to Physico chemical analysis, elemental analysis by ICP-AES and XRD analysis.⁵ The results are tabulated in table 1, 2 and 3

Table 1: Physical constants of G.R.

pH	5.54
Ash value	32.20%
Water insoluble ash	5.27%
Acid insoluble ash	3.78%
Specific gravity	0.996%
Moisture content	2.48%

Phytochemical constituents

Carbohydrates, Tannins and Steroids present

Table 2: Analysis report of Shodhita Gandhaka and Gandhaka Rasayana by ICP-AES

Components	S.G	G.R
Zinc as Zn	11.13	10.32
Iron as Fe	88.70	433.89
Copper as Cu	7.73	32.77
Nickel as Ni	N.D	10.32
Calcium	0.026%	0.84%
Lead as Pb	N.D	N.D
Sodium as Na	89.43	0.17%
Arsenic as As	67.99	12.14
Hg	-	-

Result is expressed in microgram/gm (PPM) or % as indicated.

ND for above elements means less than 0.1ppm

Table 3: XRD reports of Shodhita Gandhaka & Gandhaka Rasayana.

Samples	Cell structure	Cell volume
Shodhita Gandhaka	Monoclinic and triclinic	1033.501 A units
Gandhaka Rasayana	Triclinic	1021.082 A units

Experimental study⁶

The Antibacterial and antifungal analysis was carried by cup-plate method. The method depends on the diffusion of the drug from a cavity through the solidified agar layer of a petridish to an extent, such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of the drug.

The study was carried out taking three different concentrations of GR dissolved in solvent ethyl alcohol + water (5:95), Fluconazole as standard and ethyl alcohol + water (5:95) as control. It was tested on 3 strains of bacteria *Staphylococcus Aureus*, *Pseudomonas auregenosa*, *Escheria coli* and 4 strains of Fungi *Candida albicans*, *Cryptococcus neoformans*, *Trycophytum rubrum* *Aspergillus niger*.

The solubility of GR was found to be 76% in distilled water and ethyl alcohol (95:5) hence was taken as vehicle and control.

a) Preparation of test solution

2.5gms of G.R was added to 50ml of control solution, stirred well and filtered through Whatman filter paper no 42. Filtrate solution of G.R was taken as 100% solution. 1 ml contains 38,000 µg/ml of drug.

1ml of 100% solution of G.R. was added to 9ml control → 10% solution. 1ml contains 3,800 µg/ml of drug.

2ml of 100% solution of G.R was added to 8ml of control → 20% solution. 1ml contains 7,600 µg/ml of drug.

a) Preparation of control solution

95ml of D.W + 5ml E.A was mixed together to prepare control solution

c) Preparation of standard solution

100mg Fluconazole tablet was dissolved in 100ml distilled water and used as standard drug for antibacterial and antifungal activity. 1ml contains 1000 µg/ml of drug.

Preparation of Agar plates:

5ml of inoculums prepared was added to 45ml of flask containing nutrient agar at 37⁰ c. This was immediately poured into a dry sterile petridish to a depth of 5mm. The petridishes were placed on a leveled surface to ensure that the layers of medium are of uniform thickness. The plates were allowed to solidify at room temperature for 12hrs. Some plates were incubated at 35⁰c to check sterility. The surface of the agar layer was

kept dry before use. With the help of sterile borer (diameter 8mm) cylinders were made in agar plates. A uniform volume (i.e 0.5ml) of test solutions Gandhaka Rasayana 19,000 µg, 1,900 µg, 3,800 µg, control 95:5 of D.W + E.A and standard drug Fluconazole 500 µg were added to each cavity, sufficient enough to fill the holes.

After 30min agar plates were incubated at 37⁰c for 72hrs. Zone of inhibition was measured after 72hrs using mm scale. The diameter of the circular zone is the measurement of the zone of inhibition. The results are tabulated in table no 4

Table 4: Zone of Inhibition (in mm) of 10%, 20%, 100% G.R solution, control and Fluconazole
Antimicrobial sensitivity testing of different concentrations of G.R, standard, control in mm

Test drugs	Test Organisms						
	S.A	P.A	E.C	T.R	A.N	C.N	C.A
I. 10% G.R1900 µgm	18	14	16	12	18	12	10
II. 20% G.R3800 µgm	22	18	20	16	20	14	14
III. 100% G.R 19000 µgm	30	28	26	22	25	18	16
IV. Fluconazole	-	-	-	20	22	22	20
V. Control	10	10	12	8	10	8	10

Discussion

Gandhaka Rasayana was undertaken for antibacterial and antifungal study and tested against bacteria like Staphylococcus aureus, Pseudomonas auregenosa and E-coli and fungi like Trycophytum rubrum, Aspergillus Niger, Cryptococcus Neoformans and Candida albicans. It was found that with different concentrations of Gandhaka Rasayana, the zones of inhibition were found to be significant in bacteria compared to fungi. Significant zone of inhibition was noted in bacteria Staphylococcus aureus compared to Pseudomonas auregenosa and E-coli. Significant zone of inhibition was observed in fungi Aspergillus Niger when compared to Trycophytum rubrum, Cryptococcus Neoformans and Candida albicans. Standard drug was sensitive to all fungal organisms and highly sensitive against Aspergillus Niger and Cryptococcus Neoformans, where zones of inhibition were 22 mm. Control group has also shown sensitive to all organisms. 100% solution of Gandhaka Rasayana has shown significant zone of inhibition in comparison with 20% and 10% respectively. Gandhaka Rasayana solution in higher concentration showed similar antifungal activity compared to Fluconazole against Candida albicans and Cryptococcus neoformans, but it was more significant against Trycophytum rubrum and Aspergillus Niger than Fluconazole. With different concentrations of Gandhaka Rasayana the zones of inhibition were significant in comparison to control. As already been described, Gandhaka Rasayana consists of Sulphur along with other herbal ingredients. Gandhaka is mentioned as Krimighna in ancient Ayurvedic texts. Sulphur has been associated as an important constituent in Sulphonamides, which are used as antimicrobial agents. These groups of drugs have been proved to act by inhibiting Folic acid metabolism in the susceptible bacteria and preventing their growth.⁷ Thus Sulphur in Gandhaka Rasayana also might have same mechanism of antimicrobial activity. Sulphur

ointment topically was widely used as a scabicide and pediculocide, because one of the metabolite of Sulphur i.e. Pentathionic acid, which was suppose to cause lysis of cuticle. This proves that Sulphur is effective both systematically and topically as a microbicidal agent.⁸ In Gandhaka Rasayana, Sulphur has been detoxified with ancient process mentioned in Rasashastra text, so that the irritant and toxic effect of Sulphur is reduced. At the same time other ingredients like Amalaki, Bibhitaki etc. have attributed additional therapeutic properties and proved to have antimicrobial activity. In Amalaki, phyllimblin from stem gall callus is said to have antimicrobial property, Bibhitaki has antibacterial and antifungal property, powdered fruit of Haritaki have wide antibacterial and antifungal spectrum, oil from floral buds of Nagakeshar have proved antibacterial activity especially against Staphylococcus aureus, Guduchi has antitubercular, antidiabetic and hepato protective property and also reduces cholesterol, Bringraja has antiviral and hepatoprotective property, shunthi is antibacterial, antitubercular and improves gastrointestinal functions, Tamala is antidiabetic.⁹

Sulphur as free element cannot be utilized by the body, but is ingested as organic or inorganic sulphates. In Gandhaka Rasayana, when Sulphur is given bhavana it may be forming organic or inorganic sulphates, thus increasing its rate and extent of absorption.^{10,11} The reduction in percentage of Sulphur from 98.24% in Shodhita Gandhaka to 5.62% in Gandhaka Rasayana is in permissible amount and sufficient enough to carry out the functions like detoxification and other metabolic activities. Elements like Iron (433.84), Cu (32.77), Ni (10.32), Zn (10.32), Ca (0.84%), Na (0.17%), As (12.14) were noted in G.R. Iron helps in formation of Hb, where as Cu acts as a catalyst in Hb formation and also helps growth, gastrointestinal and reproductive functions, Ca is the main constituent of bone and teeth and also help in blood coagulation and muscle concentration, Na plays an

important role in electrolyte balance, Zinc aids in protein metabolism and plays important role in wound healing and spermatogenesis.^{10,11} As Gandhaka Rasayana contains Triclinic unit cell with average cell volume of 1021.082 Å units, it may contribute for better absorption and bioavailability. Phytochemicals like carbohydrates, steroids and tannins were present in Gandhaka Rasayana. Carbohydrates act as antidiarrhoeal and plays important role in wound healing. Tannins are astringent, cardio tonic and useful in skin eruptions boils and diarrhea. Steroids regulate carbohydrate and protein metabolism and possess strong anti-inflammatory action. They also influence the electrolyte and water balance of the body.^{12,13} Sulfonamides and Fluconazole act only as antimicrobial agents and may produce adverse effects on human beings but Gandhaka Rasayana not only act as antimicrobial agent but have additional properties like rejuvenation and promotes positive health and vigor by increasing the immunity, thus making the body resistant against disease causing factors.

Conclusion

Gandhaka Rasayana has demonstrated both antibacterial and antifungal activity but antibacterial activity was significant than antifungal activity. Gandhaka Rasayana solution in higher concentration showed similar antifungal activity compared to Fluconazole against *Candida albicans* and *Cryptococcus neoformans*, but it was more significant against *Trypophyllum rubrum* and *Aspergillus Niger* than Fluconazole. With different concentrations of Gandhaka Rasayana the zones of inhibition were significant in comparison to control. The above study has contributed for the evidence base to rationality of using Gandhaka Rasayana as antibacterial and antifungal drug (Krimighna). Gandhaka Rasayana has demonstrated significant antibacterial and antifungal activity, which gives further scope for experimental and clinical study on various microorganisms.

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