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Research Article

STUDIES ON ANTIVIRAL ACTIVITY OF *TULSI* (*OCIMUM SANCTUM*) CRUDE EXTRACTS ON SELECTED VIRUSES OF VETERINARY IMPORTANCE

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ABSTRACT

Viral diseases are the major causes of devastations in the human history and animal farming worldwide. Bacterial and parasitic diseases have been controlled by use of effective disinfectants, antibiotics and antiparasitic agents. Since, viruses are intracellular and any intervention will affect the cellular metabolism of the host, development of antiviral drugs is a challenge. Drugs acting on microbial agents have been mentioned in Ayurvedic texts as *Krimighna Dravyas*. *Tulsi, Ocimum sanctum* is one of the most important medicinal plants mentioned in Ayurvedic literature for its medicinal and spiritual properties. The plant is an highly celebrated medicinal plant as "The incomparable one," "Mother medicine of nature" and "The queen of herbs". *Tulsi*, along with other health benefits is known to have anti-infective functions. Hence, antiviral activity of aqueous, ethanol, methanol and chloroform extract of powdered drugs was evaluated against economically important viruses of veterinary importance, Orthmyxovirus and Paramyxovirus. The *in vitro* cytotoxicity confirmed the safety of the extracts and aqueous extract showed no inhibition on paramyxovirus while showing moderate inhibitory activity on orthomyxovirus while ethanol extract showed moderate inhibitory activity on paramyxovirus and no activity on orthomyxoviruses. Methanol extract showed no inhibition on of paramyxovirus while showed significant inhibition of orthomyxovirus. Chloroform extract of the plant showed no inhibition paramyxovirus while significant inhibition was observed on orthomyxoviurs. Results of the study suggest that the *O. sanctum* can be used as antiviral agent for effective control of viral infections of animal importance.

KEYWORDS: *Tulsi*, *Ocimum sanctum*, Antiviral Activity, Orthomyxovirus, Paramyxovirus.

INTRODUCTION

Viral infections continue to be major threat for human and animal health significance worldwide including India. Infectious nature of viral pathogens, eluding host-viral pathogen and non-availability of cost effective antiviral molecules have further complicated the therapeutic management of viral diseases both in medical and veterinary practice. Though the search for new antivirals is in progress natural molecules from plant sources have the immense potential to be developed as effective antiviral products. Identification of newer bioactive molecules from fungi, marine fauna and flora, bacteria and plants sources is being done extensively throughout the world. To enhance the probability of identifying the therapeutically effective molecules, studying the ethnopharmacological knowledge available with traditional healing systems of ancient civilizations including Indian Ayurveda is the most suitable approach. However, the medicinal plants mentioned in Ayurvedic texts need to be evaluated using modern *in vitro* assays.

Unlike other pathogens, viruses are obligate intracellular parasites use host cells for all its metabolic activity and replication. Hence any antiviral drug will have detrimental effect on host cellular metabolism posing difficulty in designing drugs acting on specific metabolic pathways of the virus replication. This has diverted the attention of researchers to develop antiviral agents from their native traditional plant medicines. Drugs acting on microbial agents have been mentioned in Ayurvedic texts as *Krimighna Dravyas. Tulsi*, *Ocimum sanctum* is an highly celebrated medicinal plant as "The incomparable one," "Mother medicine of nature" and "The queen of herbs," and is regarded as an "elixir of life" that is without equal for both its medicinal and spiritual properties. [1]

Promising novel antiviral phytochemicals from plant sources like, flavonoids, terpenoids, lignans, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides have been

reported recently. It is interesting to note that some of the commonly used culinary herbs, spices and herbal teas have also known to exhibit high level of antiviral activity. Medicinal value of *Tulsi* has been extensively described in Ayurvedic literature for treatment of worms,[2-3] cough,[4] urticaria,[5] grey hairs[6] and wounds.[7] Recently there is a renewed interest in medicinal plants of *Ocimum* spp as mosquito repellent and as anti-infective agent.[8-10] Antimicrobial activity of medicinal plant *Ocimum sanctum* has been reported extensively.[11-14] Various pharmacological activities of the plant like, adaptogenic, protection and detoxification, improving metabolism, protection against infection, anti-stress effects have been reviewed extensively[15]. Since several of important viral pathogens of humans belong to paramyxo and orthmyxo virus family this communication reports the antiviral effect of *Tulsi* (*Ocimum sanctum*) crude extracts on similar viruses of veterinary importance. Orthomyxovirus and Paramyxovirus were selected as representative viral agents as they represent important group of viruses causing economically important and zoonotic diseases of domestic animals and poultry.

MATERIALS AND METHODS

Preliminary phytochemical analysis

Dried and powdered *Tulsi* (*Ocimum sanctum*) leaves (Fig.1) were subjected for phytochemical analysis like, detection of tannins, alkaloids, saponins, cardiac glycosides, anthroquinone glycosides, steroids, resins and volatile oils for qualitative assessment of phytoconstituents.[16]

Extractions from the powdered plant material

Extraction of active constituents from powdered *Tulsi* (*Ocimum sanctum*) was done as given below. Twenty grams of air dried powdered leaf was subjected to soxhlet apparatus for 12 hr for aqueous extraction and 100 grams of the powdered drug was used for ethanol, methanol and chloroform extraction by cold percolation on magnetic stirrer for 24 h in ten times its volume in 80% ethanol, methanol or chloroform respectively. The extracts were clarified by filtration first through double layer of muslin cloth and then through Whatman filter paper No.1. Yield of the extraction was calculated after drying the filtrate under low heat of 50^0 C. The extracts was stored at -20^0C till further use after diluting to the predetermined concentration.

Testing the antiviral activity

Cell culture, embryonated hen's egg and viruses

Bovine kidney cells (MDBK cell line) and African Green Monkey cells (Vero cell) were grown as a monolayer culture in Eagles minimum essential medium (MEM) supplemented with 10% foetal bovine serum (FBS), 100 units/ml penicillin and100 ug/ml streptomycin. The cultures were maintained at 37 ^{0}C in a humidified 5% CO_2 incubator. Viruses were maintained in 10-11 days old embryonated hen's eggs by inoculating *Via* allantoic route of inoculation. Animal viruses Orthomyxovirus and Paramyxovirus were used for testing the antiviral activity of crude extracts of *Tulsi*.

Cytotoxicity assay

The dry crude extracts of *Tulsi* were re-dissolved in dimethyl sulfoxide (DMSO) and 10 fold dilutions were made in cell culture medium. Adherent cell monolayers were trypsinized and cells were plated at 15,000/well in 96-well flat-bottomed plates for tetrazolium-dye (MTT) cytotoxicity assay. Following incubation for 24 hr, dilutions were added to the appropriate wells and the plates were again incubated for 24 and 48 hrs at 37^0C in a humidified CO_2 incubator while untreated cells were used as controls. Supernatants were removed from all wells and 25 ul of MTT (2mg/ml) solution in phosphate buffer saline (PBS) was added and the plates were incubated for 2 hr at 37^0C and 125 ul of DMSO was added to the wells to stabilize the MTT crystals. The plates were placed in shaker for 15 min and absorbance was read at 492 nm. Spectrophotometer blank were the cells lysed with DMSO 2 hr previous to evaluation of cellular viability by the MTT assay. The percentage of cytotoxicity was calculated as (A-B)/A x 100, where A is the mean optical density of untreated wells and B is the optical density of the wells with plant extract.

Antiviral assay

To screen the antiviral activity dilutions of the *Tulsi* extracts were preincubated with the standard concentration of virus (orthomyxovirus and paramyxovirus) for one hr in shaker incubator at 37^0C and were inoculated (0.1 ml) through intra-allantoic route to 10-11 days old embryonated hen's eggs. Holes were sealed and eggs were incubated for 48 hrs in humidified incubator of 37^0C. Extracts, viruses and medium were kept as control. The observations for antiviral activity was recorded after 48 hrs by observing the survivability of embryos in the inoculated eggs and checking the embryo fluid by haemagglutination test using 1% chicken red blood cells.[17]

RESULTS AND DISCUSSION

Results of the qualitative phytochemical analysis for tannins alkaloids, saponins, cardiac glycosides, steroids: trpenoids and flavonoids confirmed the authenticity of the medicinal plant *Tulsi*, *O. sanctum* (Table 1). The *in vitro* cytotoxicity of the crude plant extracts was evaluated using MDBK cells and compared with standard controls. The IC_{50}

values in mammalian cells indicated no cytotoxicity effect in the cells evaluated and found to be safe for use.

Aqueous extract of the plant showed no inhibition on paramyxovirus while showing moderate inhibitory activity on orthomyxovirus tested in the present study while ethanol extract showed moderate inhibitory activity on pramyxovirus and no activity on orthomyxoviruses. Methanol extract showed no inhibition of paramyxovirus while showed significant inhibition of orthomyxovirus. Chloroform extract of the plant showed no inhibition paramyxovirus while significant inhibition was observed on orthomyxoviurs (Fig.2, Table 4).

Bioactive principles identified in *T*ulsi were eugenol, citrusin, ferulaldehyde, bieugenol, dehydrodieugenol, oleanolic acid, ulsoric acid, stigmasterol, b -sitosterol-3-O-b –D glucopyranoside, caryophyllene oxide, apigenin, luteolin, crysoeriol, 5-dihydroxy-7,8-dimethoxyflavone, 5-dihydroxy-3, 7,8-trimethoxyflavone and vanillin.[18] Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the major active constituent present in *O. sanctum*, has been found to be largely responsible for the therapeutic potentials. Over all observation indicate the further intense research work is required to standardize the dose and concentration of the drug. Future investigations need to orient towards identifying the active principles responsible for antiviral properties for successful clinical applications both in medical and veterinary practice.

Table 1: Phytochemical analysis of *Tulsi (Ocimum sanctum)*

Alkaloids	Glycosides	Tannins	Saponins	Steroids	Flavonoids	Anthra quinones	Volatile oils	Resins	Starch
+	+	+	+	-	-	-	+	-	-

Table 2: Antiviral activity (percentage inhibition) of different crude extracts of *Tulsi (Ocimum sanctum)* on selected animal viruses

Sl.No.	Plant material	Paramyxovirus	Orthomyxovirus
1.	Aqueous	0%	50%
2.	Ethanol	50%	0%
3.	Methanol	0%	75%
4.	Chloroform	0%	75%
5.	Virus control	0%	0%
6.	DMSO control	0%	0%

Table: 3. Cytotoxic assay results (percentage toxicity) of different crude extracts of *Tulsi (Ocimum sanctum)* on MDBK cells

	24 hr post inoculation								48 hr post inoculation							
Dilution →	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Extracts																
Aqueous	38	38	37	37	36	35	35	35	44	44	44	44	43	43	42	41
Ethanol	36	36	35	35	34	34	34	31	45	45	44	44	44	43	43	40
Methanol	35	35	34	34	34	33	33	33	47	47	43	43	43	42	42	42
Chloroform	39	39	38	38	36	36	35	35	48	48	46	46	45	45	44	44
Virus control	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
DMSO control	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Fig.1. Tulsi (*Ocimum sanctum*) plant and the dried leaf powder

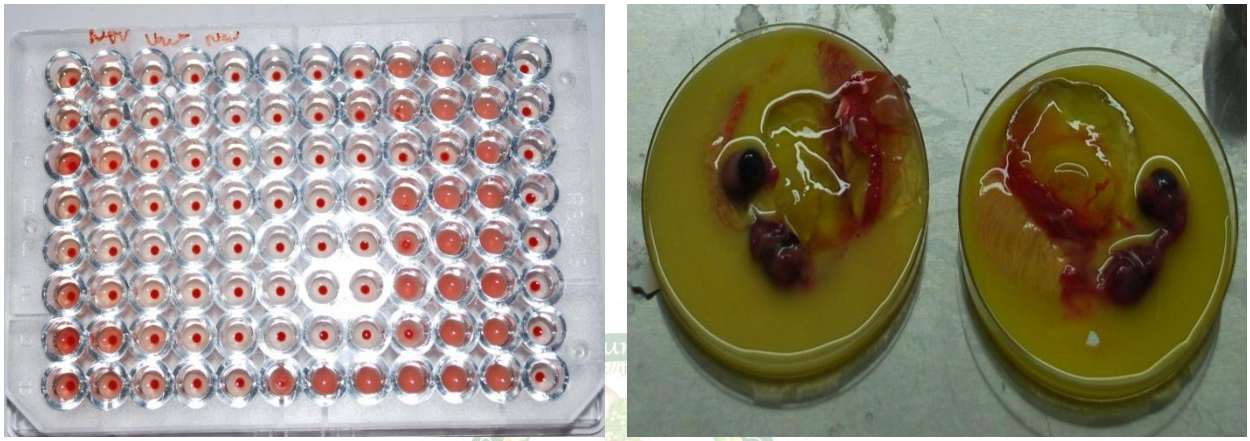

Fig.2. Haemagglutination activity of the orthomyxo and paramyxovirus, haemorrhages in the chicken embryos

REFERENCES

1. Singh N, Hoette Y, Miller R. Tulsi: The Mother Medicine of Nature. 2nd ed. Lucknow: International Institute of Herbal Medicine; 2010. p. 28-47
2. Sharma, P.V. Astanta Hrdaya Chikitsastana 20/27, In Classical Uses of Medicinal Plants, 2004 Chaukhambha Visvabharati Publications.
3. Sastri, K.A, Susruta Samhita Sutrasthana. 38/18-19, Chaukambha Sanskrit Sansthan, Varanasi; 2012.
4. Sharma, P.V. Caraka Samahita.Chikitsastana 18/117, In Classical Uses of Medicinal Plants, 2004 Chaukhambha Visvabharati Publications.
5. Suresh Babu, M.S. Yoga Ratnakara, Chowkhamba Sanskrit Series Office; First edition pp 348
6. Sharma, P.V. Astanga Hrdaya Uttarasthana 24/37, In Classical Uses of Medicinal Plants, 2004 Chaukhambha Visvabharati Publications.
7. Sharma, P.V. Vrinda Madhva, 44/44, In Classical Uses of Medicinal Plants, 2004 Chaukhambha Visvabharati Publications.
8. Singh, S., Mahour, K., Prakash, S., Evaluation of mosquito repellent efficacy of *Ocimum sanctum* plant extract. Journal of Herbal Medicine and Toxicology, 2009; 3(1):87-90.
9. Dey, B.B. and Chodhary M.A., Essential oil of *Ocimum sanctum* L. and its antimicrobial activity. Indian Perfumer, 1984; 28:82-87.
10. Tripathi R.K.R and Tripathi R.N., Reduction in bean common mosaic virus (BCMV) infectivity vis-a-vis crude leaf extract of some higher plants. Experientia, 1982; 32 (3):349.
11. Rani, P. and Khullar, N., Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytotherapy Research, 2004;18(8):670-673.
12. Mukherjee, R., Dash, P.K. and Ram, G.C. (2005). Immunotherapeutic potential of *Ocimum sanctum* (L) in bovine subclinical mastitis. Research in Veterinary Science, 2005; 79 (1): 37-43.
13. Singh, S., Malhotra, M. and Majumdar, D.K., Antibacterial activity of *Ocimum sanctum* L. fixed oil. Indian Journal of Experimental Biology, 2005; 43:835.
14. Singh, S., Taneja, M. and Majumdar, D.K., Biological activities of *Ocimum sanctum* L. fixed

oil-an overview. Indian Journal of Experimental Biology, 2007;45:403-412.

15. Cohen, M.M., Tulsi - *Ocimum sanctum*: A herb for all reasons. Journal of Ayurveda and Integrated Medicine, 2014;5:251-259.
16. Trease, G.E. and Evans, W.C., Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. 1983. pp. 343-383.
17. Swayne, D.E., D.A. Senne and C.W. Beard, Avian Influenza, in a Laboratory Manual for the Isolation and Identification of Avian Pathogens. 1998. 4th Edn., American Association of Avian Pathologists, Kennett Square, USA.
18. 18. Suzuki, A., Shirota, O., Mori, K., Sekita, S., Fuchino, H., Takano A, et al., Leishmanicidal active constituents from Nepalese medicinal plant Tulsi (Ocimum sanctum L.). Chem Pharm Bull (Tokyo), 2009;57:245‑251.

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