A Systematic Review on Design Development and Evaluation of Herbal eardrops

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Abstract: An ear infection affects the middle ear; they are more common in children. Unlike the synthetic drugs, the herbal products have a complex composition, being complex mixtures of bioactive compounds. The main aims of this review were to develop a novel ear drop solution based on different phytocompounds and to characterize it by specific methods. Avurveda prescribes instillation of medicated oil and juice of certain medicinal plants in the affected ear. An increase in multi drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases which has triggered immense interest in the search of new drugs or preparations from the natural sources including plants. There is a great structural diversity exist among antimicrobial phytocompounds. This paper describes the obtaining and the preliminary characterization of mixed solutions based on herbal extracts.

Keywords: Anti-microbial, Anti-inflammatory, Herbal Eardrops, Plant extracts.

INTRODUCTION

The ear serves a crucial purpose in balance and spatial orientation in addition to communication and socialising with other people. There are various parts to the acoustic vestibular system, including a peripheral segment made up of the outer, middle, and inner ears. A painful sensation located in the auricle, ear discomfort can be pressing, stabbing, stinging, throbbing, etc. and can vary in intensity. According to where the inflammation is, there are three main categories of otitis: external, middle, and internal. An external auditory canal or ear lobe infection is known as otitis externa (the canal leading to the eardrum). Otitis media is an inflammation of the tympanic cavity and eardrum (a small chamber, located behind the eardrum, normally full of air, which in otitis media fills with fluids - serum, mucus or pus). Cochlear and vestibular labyrinth inflammation is a less frequent component of otitis media. [1],[2],[3]

Man has always used plants for healing and almost as soon as he learned to write he recorded descriptions of their healing properties in different "handbooks on plants". The first known data was written almost 5,000 years ago during the Chinese Emperor Chi'en Nung; it was called Pen Tsao and it contains the descriptions of the medicinal uses of over 300 plants. By 2000 BC, the ancient Egyptians used plants in medicine, cosmetics and embalming; Greeks and Romans have perfected some of these techniques and developed new ones of their own. They learned about their studies from the writings of Hippocrates in the fifth century BC and from the books "De Materia Medica" by Dioscorides and "Naturalis Historia" - 37 volumes by Pliny the Elder (both from the first century AD). [4],[5],[6] One of the biggest dangers to the effective treatment of microbial diseases is the rise of multidrug resistant infections, which has sparked intense interest in the development of novel medications or preparations from natural sources, including plants. A huge number metabolites of its secondary demonstrated antimicrobial activity. Medicinal plants have a widespread antibacterial action. The structural diversity of antibacterial phytocompounds is substantial. Alkaloids, anthraquinones, cardiac glycosides, saponins, tannins, and polyphenols are among the major categories of phytocompounds. Ear pain is an extremely painful symptom that may be brought on by internal ear reasons or external sources.

THE GENERAL CAUSES OF EARACHE ARE AS FOLLOWS

The earache may increase on lying down due to increased blood supply to the ear in recumbent

position. [7],[8]

exposure to very cold climate. diving or swimming under pressure fluctuations, flying, picking or prodding the ear canal. incorrect apparatus used for examining or treating ears. exposure to noises with a very high pitch. Head injury, particularly to the temporal bone. water entering the auditory canal when bathing. localised bacterial or fungal infection, including otomycosis and furuncles. affected wax referred pain brought on by a carious tooth, an impacted molar, inflammatory lesions in the mouth and tongue, temporomandibular joint osteoarthritis, tonsillitis, etc. The earache can also be functional, which needs to be carefully observed. Management of earache requires proper evaluation and treatment before complications develop. Ayurveda prescribes instillation of medicated oil and juice of certain medicinal plants in the affected ear. This paper describes the obtaining and the preliminary characterization of mixed solutions based on herbal extracts, that can be used in the otitis treatment.[9]-[13]

MATERIAL AND METHODS

The preparation of eardrops having following materials and methods such as Plant Collection, Extraction of plant material, Test organisms, Preparation of eardrops, Screening for activity, Determination of minimum inhibitory concentration (MIC), Statistical analysis.

Plant Collection:

Collection of Plants species and Identification of parts for e.g. roots, leaves, stems, barks, etc.

Taxonomical Study of plant is carried out in this.

Extraction:

The samples were carefully washed under running tap water followed by sterile distilled water and air dried at room temperature (40°C) for 4-5 days and then homogenized to a fine powder using a sterilized mixer grinder and stored in airtight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction.

Test microorganisms

Some bacteria like Staphylococcus aureus (HM626197)* (Gram-positive), Acinetobacter sp. (HM626198), Proteus mirabilis (HM626199), Escherchia coli (HM626200), Pseudomonas aeruginosa (HM626201) (Gram-negative) and one

yeast, Candida albicans used in this study, were isolated from the patients having ear infection from the local ENT clinics in particular areas.

Bacterial strains were identified on the basis of staining, biochemical and molecular characteristics (16S rRNA sequencing)33 and yeast on the basis of staining, morphological and cultural characteristics 5,34,35. The bacterial isolates were subcultured on Nutrient agar and yeast on Malt yeast agar and incubated aerobically at 370 C. The media were procured from Hi Media Laboratory Pvt. Ltd., Bombay, India.

Preparation of Ear Drops:

Aqueous solutions (1:100, w/v) of vegetal extracts were prepared to evaluate their properties, their toxicity, and their efficacy; a sterile saline solution (9 mg/mL NaCl) was used as the solvent. Polypropylene sterile vials containing these solutions were kept at room temperature before any evaluation. [14],[15]

PHARMACOLOGICAL SCREENING

1) Anti-inflammatory:

Any drug or substances reduces inflammation are called as anti-inflammatory agents. These can block certain substances in ear that causes inflammation. There are three major groups of otitis, corresponding to the location of inflammation: external, medium and internal. The popular murine macrophage cell line, RAW 264.7, is often used to initially screen natural products for bioactivity and to predict their anti-inflammatory effects. an ear solution based on a mixture of Aloe vera and Tea tree extract can be used in the management of otitis. [14]

2) Anti-microbial:

The antimicrobial activity of fruit extracts were tested by agar well diffusion method. Against the six ear pathogens causing ear infection namely, *E.coli, Candida albicans, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* in different solvents. Organic fruit extracts displayed antimicrobial activity against five tested ear pathogens and aqueous extracts were unable to exhibit any antimicrobial activity.[15]

3) Anti-bacterial:

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An ear infection is caused by a bacterium or virus in the middle ear. They often result from a blockage of your Eustachian tubes, which causes fluid to build up in your middle ear. particularly the bacteria *Streptococcus pneumoniae* Trusted Source or *Haemophilus influenzae*. It can be treated by using some drugs like Allium Sativum, Brassica compestris Linn. Acacia nilotica.

4) Anti-fungal:

Fungal ear infections usually cause a fair amount of discomfort and discharge. 9 out of 10 fungal infections are due to a fungus belonging to the *Aspergillus* species and the rest are caused by a fungus of the *Candida* species. the infection should respond fairly quickly to antifungal medications such as Allium Sativum, Brassica compestris Linn., Acacia nilotica, etc. [17],[18]-[21]

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC for each test organism was determined by following the modified agar well diffusion method. A twofold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg/ml) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100ul of standardized inoculum (106 cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 370 C for 24 hrs and observed for the inhibition zones. MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>8mm), was recorded for each test organism. [14]

Statistical analysis

All data were presented as mean \pm SD (Standard deviation). Results were statistically evaluated using Dennett's T-test. P value less than 0.01 were considered significant.

Characterization of Ear Drops:

Ear drop is characterized by using parameters like. pH , Absorbance , Screening of Phytochemicals , Invitro Screening , Efficacy evaluation, etc.

pH:

The pH values of the samples based on the ear drops solutions were determined using a HI 2221 (Hanna Instruments, USA) with a combined electrode (a glass electrode and a calomel reference electrode) at 25°C.

Absorbance:

It describes the results of the UV-Vis characterization. Different absorption levels were found for the investigated compounds.

Efficacy evaluation:

The murine macrophage cell line RAW 264.7 was achieved from the American Type Culture Collection (ATCC, USA). The cells were cultured in Dulbecco's modified Eagle's medium. This method assesses the activity of mitochondrial dehydrogenase from metabolically viable cells. The effect can be quantified through a colorimetric reaction in which the MTT (yellow compound) is reduced by viable cells to formazan (dark blue compound) according to a previous study.[14]

Evaluation of Eardrops:

Ear drop is evaluated by using parameters like, foreign particles, Sterility, pH, Viscosity, etc.

Foreign particles:

All product should be clear and free from foreign particles, fibers, filaments, etc. these solutions should be clarified very carefully by passing through bacteria proof filters such as membrane filters, sintered glass filters.

Sterility:

Dry heat sterilization at 160° c for 2 hours for non-aqueus solutions. Autoclaving at 115° c for 30 min and 121° c for 15 min.

pH:

pH plays an important role in therapeutic activity, solubility, stability and comfort to the patients. Ear drops having pH level maintained at 6-7. [16]

Table 1 : Plants used for preparation of herbal eardrops

Sr. No.	Name of Plant Used	Materials	Eardrops (oil/water based)	Activity	Reference No.
1	Cassia fistula	Ethanol/Methanol/Acetone/ Water(Hot/Cold)	Oil	Anti-microbial	[20]
2	Elettaria Cardamomum	Ethanol/Methanol/Acetone/ Water(Hot/Cold)	Water	Anti-microbial	[15],[18],[19]
3	Terminalia Arjuna	Methanol/Ethanol/ Acetone/Water(Hot/Cold)	Water	Anti-microbial	[21-31]
4	Tulsi Oil (Ocimum spp.)	Water extract	Water	Anti-microbial	[32]
5	Terminalia chebula	Methanol/Ethanol/ Acetone/Water(Hot/Cold)	Water	Anti-microbial	[33]
6	Camphor with fresh coriander juice	Camphor with fresh coriander juice	Water	Anti-microbial	[34]
7	Calandula officianalis (marigold)	Water extract or Oil infusion	Oil	Anti-microbial Anti- inflammatory	[35]
8	Allium Sativum , Brassica compestris Linn.	Alcohol/Water	Water	Anti- inflammatory Anti-fungal Anti-bacterial Anti-viral Anthelmentic	[36]-[41]
9	Acacia nilotica	Methanol/Ethanol/ Acetone/Water(Hot/Cold)	Water	Anti-microbial Anti-fungal An11ti- bacterial	[42]
10	Aloe vera and Tea tree	Ethanol	-	Anti- inflammatory	[14],[43]- [45]
11	Phajus flavus (Bl.) Lindl.	Water extract or Infusion	Oil	Pain reliever	[46]
12	Xanthium strumarium L.	Water extract or Infusion	Oil	Pain reliever	[46]

CONCLUSION

Otitis media can affect people of any age. It is typical symptoms include pressure sensation, ear pain and hearing loss; the existing treatments are effective with a good chance of cure, but also with the help of some natural methods. In this study, different samples based on herbal extracts have been obtained and characterized. The comparative analysis revealed that the best effects were obtained in the case of the samples. The antimicrobial activity of fruit extracts against the tested ear pathogens may be due to the presence of secondary metabolites mainly, terpenoids belonging to the class of monoterpenes (linalyl acetate, nerol, neryl acetate, geranyl acetate, geraniol, citronellol, linalool, cisocimene and methylheptenone) and sesquiterpenes (t:caryophyllene, valencene, Nerolidol, farnesol).

At last, the need of the hour is to perform more and more screening of the natural products or plant parts as such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antifungal and antibacterial compounds against the ear pathogens and the resistant bacterial and fungal pathogens. we still need more detailed studies as *in vivo* testing and pharmacokinetics properties for their therapeutic utility in treating ear infections.

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