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CALRD is working on a mission to promote research and development culture in Pakistan. RBS2021 is the part of out 100 seminar project.

We hope during your time at the conference that you take the opportunity to engage with your peers to discuss your ideas for research and practice and that you ask questions of the presenters. There will be plenty of opportunities for collaboration.

Dr. Rao Kashif

CEO/Managing Director

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Pakistan

# **Keynote Speakers**

# Dr. Faiza Rao

Assistant Research Professor

Managing Director

Center to Advance Level Research and Development (SMC-PVT) Ltd

### **Topic**

Importance of Endometrial Decidualization

## Dr. Chan Zhou

Postdoctoral Researcher Center for Reproductive Medicine, Shandong Provincial Hospital, Shandong University, Jinan, China

### Topic

Sequential Activation of Uterine Epithelial IGF1R-STAT3 Signaling by Stromal IGF1 and Embryonic IGF2 Conquer the Epithelial Barrier for Successful Implantation

# Dr. Shazia Perveen

Assistant Professor
The Women University Multan
Punjab, Pakistan

# Topic

Role of Polyphenol in Neuronal Disorders and Cancer

# Dr. Anam Rao

Medical Officer Multan Institute of Kidney Diseases (MIKD) Punjab, Pakistan

## **Topic**

Kidney Health Issues

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# **Evaluation of Biological Properties of Pure Biofine Chemicals and Aroma Substances From Cannabis Sativa Essential Oil**

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#### **Abstract**

Essential oils are a mixture of bioactive chemicals that are useful to humans as therapeutics and preservatives. The essential oils of Cannabis sativa have been tested for their antioxidant, fungicidal, bactericidal and cytotoxic properties. A maximum of 0.035% extraction was found at 110°C. Caryophyllene, trans-α-bergamotene, humulene, and cis-β-farnesene in different percentages were major components to more than 29 major and minor identified in each oil of C.sativa via GC-MS analysis. Separation is done using a fractional method. The total phenolic content was found to be 13.42-13.83 and 11.24-14.68 mg GAE/ml; the total flavonoid content was 14.12-14.22 and 10.78-15.39 mg CE/ml, and the estimated content of proanthocyanidin was 8.91-10.46 and 7.16-11.31 mg VE/ml of C.sativa essential oil and fractions, respectively. Antioxidant activity was tested with DPPH• intake of 20.55-26.26% essential oil and 37.77% high in F1 of 110°C essential oil. The ability to reduce ferric ions was set at 2.4-2.69 mg/ml, and the maximum reduction potential of 2.78 mg/ml was F5 of 120°C essential oil at 0.3 mg/ml concentration. The high inhibition of linoleic acid peroxidation was shown by F4, 25.67% of 130°C extracted by the essential oil. Antibacterial values 19.33mm, 19.67mm, 19.82mm zone of inhibition and antifungal activity 20.15mm, 22.13mm, 22.26mm zone of inhibition indicated by F1, F5 and F4 of 110°C, 120°C and 130°C extracted oil Cannabis essential oils are sequential. Similarly, MBC and MFC are also shown with these components. The concentrations of brine shrimp cytotoxicity 20ppm and 25ppm have been shown by F1 and essential oils collected at 110°C. Sativa oil controls cancer and antibacterial properties as used in medicine.

# Assessment of Bacterial Contamination on the Hands Assessment of Poultry Butchers and Determination of Mdr Resistance Salmonella

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#### **Background**

Hands play a vital role in the prevention of infectious diseases as most of them are caused due to the transmission of microorganisms spread by contaminated hands.

#### **Objective**

The present study was conducted in Hyderabad and Jamshoro districts of Pakistan to assess the handwashing practices of poultry butchers using a questionnaire survey.

#### Methodology

Microbial assessment of hands of butchers was also carried out by using some standard microbiology techniques. Three hygiene indicator bacteria were targeted in this regard, namely, *Salmonella*, *Shigella*, and *E. coli*. Moreover, for *Salmonella*, antibiotic resistance was also carried out against the commonly used antibiotics.

#### Results

Survey results revealed that none of the slaughtering facilities had any hand washing station, clean rinsing water, soap/hand wash, or any other hand washing facility. Microbial analysis revealed that 35 (92.1%), 37 (97.3%), and 38 (100%) of hand samples were positive for *Salmonella*, *Shigella*, and *E. coli*. The results of the disc diffusion test revealed that 89.4% of isolates were resistant to ampicillin, 2.5% to azithromycin, 26.3% to gentamicin, 26% to cefotaxime, 34.2% to erythromycin, 27% to streptomycin, and 2.6% to sulphamethoxazole. None of the isolates showed resistance to ceftazidime. Out of 35 isolates, 25 isolates were Multidrug-resistant (MDR), and one isolate (2.85%) was extremely drug-resistant (XDR).

#### Conclusion

The study concludes the presence of *Salmonella, Shigella,* and *E. coli* in poultry slaughterhouse workers. The high prevalence of *Salmonella, Shigella, and E. coli* can transfer to the meat and cause many foodborne infections in meat consumers. Moreover, the high resistant profile of *Salmonella* spp. can cause treatment failure against *Salmonella* infections.

**Keywords:** Hands, Hygiene, Butchers, MDR, and Antibiotics

### Plants; New Therapies of endometriosis (Poster)

Anosha Khan, Rabbia Amin & Dr. Faiza Rao University of Central Punjab

One of the study conducted in 2017 concluded frquency of Endometriosis in women with infertility and sub-fertility was found to be 20%. They also found no association between demographic features agae, Parity and socio-economic class and occurrence of endometriosis.

One more study is aimed in very next year 2018 to measure the level of DEHP in women's with endometriosis and without endometriosis the results shows that high level of DEPH which is a common EDCs women's exposed by the use of cosmetics and other products are also play the role of etiology of endometriosis and permit careful use of such compound.

- Many different plants drug are used for the treatment of endometriosis. This review emphasis on the beneficial points on plants based drug such as Allium sativum, cupaifira langsdorffii, pines pin stir are checked out against endometriosis.
- There are many studies on endometriosis in Pakistan but no specific study shows the actual percentage of endometriosis in Pakistan. We need an extensive study to highlight this major disease in females.
- Therefore, we consider medicinal plants and phytochemicals excellent adjuncts in the treatment of endometriosis, but they are currently insufficient as unique therapeutic tools. The decision to consume such dietary supplements must be made with a doctor.

#### Viburnum opulus L.



Chlorogenic acid: Rats, surgically induced endometriosis used. Endometriotic volume decreases, Level of TNF- alpha, VEDG and IL-6 also decreases.

#### Allium sativum



When used in endometriotic cells it reduced cellular proliferation, induced apoptosis and Also show anti-angiogenic activity. Further show Decrease activity of VEGF and VEGFR expression

#### Curcuma longa



Rat with surgically induced endometriosis decreased volume Of endometriotic lesions, anti-angiogenic effects, anti-inflammatory effects, pro-apoptotic and antioxidant activity also

### Fish Borne Parasitic Zoonosis: Major Food Safety Concern

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#### **Abstract**

Fish is consumed raw or undercooked, as it is the tradition of most communities. Among many parasites infecting the fish, helminths belonging to the family Anisakidae, Gnathostomidae, Opisthorchiidae, Heterophyidae and Diphyllobothridae are zoonotic in nature. Over half a billion people are at risk of fish-borne helminthiasis around the globe. Intensification of aquaculture, waste disposal, environmental changes, and shorter cooking time increases the risk of zoonotic diseases. Immigration and similar demographic changes like animal transportation, global trading of fish, cultural changes in fish-eating behavior, and stocking of imported fish in new aquatic habitats are linked to new patterns of infection. The control of fish-borne zoonosis needs multidisciplinary and multisectoral participation. It can be achieved by controlled transmission of infections in the reservoir and intermediate hosts, improved fish farming practices, upgraded aquaculture systems to abolish contamination of the ponds, avoiding discard of infected fish or viscera, environmental sanitation, and mass chemotherapy of people at risk in endemic areas. It is advisable to avoid the consumption of raw, undercooked, and poorly prepared dried or pickled fish. Global food standards advocate the physical removal of parasites and freezing raw, pickled, or cured fish.

However, these practices do not put an end to parasites completely, which is uneconomical and unaccepted by consumers. The intricacy of diagnosis, complicated human cultural behaviors, and incomprehension of potential economic costs have made this field scientifically dubious and unappealing to researchers. There is room for researchers to develop specific molecular or immunological methods to aid diagnosis. Morphological identification needs to be made by molecular approaches like molecular markers, genetic markers, phylogenetic analysis to solve the long due misunderstandings of the host associations and host ranges for several species of fish helminths, wrong taxonomy, and distribution.

**Keywords:** Fish parasites, Helminths, Foodborne Zoonosis

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# Immunoprophylaxis for Control of Gastrointestinal Parasites of Animals: From Research to Commercialization

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#### **Abstract**

Gastrointestinal (GI) nematodes are considered a significant threat to small ruminant production systems in terms of high morbidity, mortality, production losses, and increased treatment cost. Traditionally, GI parasites are controlled through synthetic chemicals, biological agents, pasture and nutritional management, and selective breeding of parasite-resistant animals. However, all these have limitations in terms of low specificity and sensitivity. Boosting up of host immune system through immunoprophylaxis is a relatively sustainable method. Vaccines successfully control parasitic diseases by utilizing the host's protective immune responsiveness in order to limit the production losses and pathology. Identification of vaccine candidate/proteins antigens against non-blood feeding GI parasites of animals is still a challenge. However, much progress has been made against a model GI parasite of a small ruminant, *Haemonchus (H.) contortus*, a blood-feeding nematode.

Native antigen vaccine against *H.contortus* is effective as up to 75% reduction in fecal egg count has been reported using native antigens as larval ES antigen, cuticular collagens, and other putative antigens. Hidden antigens used against H.contortus are H11, H-gal-GP, and Cysteine proteases. Adult 15/24 kDa ES antigens and Hc-sL3 d are recently used native antigens. However, for commercial availability of vaccines, there is a significant need for antigens identification, sequencing of the genome and expressed sequence tag (EST), mRNA expression analysis, identification of signal sequences, and proteomics. RNA interference for rapid functional screening, Reverse-Transcription PCR, representational difference analysis (RDA), proteomics, and glycomics are molecular technologies used to examine the expression of genes after infection. In order to understand the molecular nature of host-parasite interaction, the use of these biotechnologies would be helpful and may lead to an innovative plan of action for parasite control. A lot of things are under consideration for designing and commercialization of vaccines. The use of vaccination against parasitic diseases of animals in veterinary health services is likely to contribute considerably to aggrandizing the productivity of livestock. Consequently, it is expected that vaccination against parasites will be used as one of the best options to control parasitic infections.

Keywords: Gastrointestinal Parasites, Immunoprophylaxis, Vaccination, Commercialization

## In Vitro Assessment of Pakistani Bee Propolis Extract Against Staphylococcus Aureus

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#### **Abstract**

Antibiotic resistance of *Staphylococcal* infections has prompted the pharmaceutical and scientific community to consider alternate treatments. Propolis is a natural substance produced by honey bees from the exudates of different plants. The aim of the current study was to evaluate the antibacterial activity of ethanolic extracts of Pakistani bee propolis against *Staphylococcus aureus*. The Propolis sample was collected from district Kohat and dried in the dark until processed. The dried propolis was dissolved in ethanol solvent and concentrated by evaporating the solvent through a rotary vacuum evaporator to obtain the final extract. The antibacterial activity of propolis extract was examined using the Agar well diffusion method. *Staphylococcus aureus* culture was incubated on MHA media. Five different concentration of propolis,  $100 \mu g/ml$ ,  $200 \mu g/ml$ ,  $350 \mu g/ml$ ,  $500 \mu g/ml$  and  $650 \mu g/ml$  were used. Gentamicin disc was used as a positive control. After 24 hours, the antibacterial activity of propolis extracts was checked, and the zone of inhibition was measured. Experiments were performed in triplicate. The mean zone of inhibition and standard deviation for each concentration were,  $17\pm0.816$  at  $650 \mu g/ml$ ,  $14.6\pm0.471$  at  $500 \mu g/ml$ ,  $12\pm1.41$  at  $300 \mu g/ml$ ,  $9.6\pm0.942$  at  $200 \mu g/ml$ ,  $2.3\pm0.471$  at  $100 \mu g/ml$  of propolis extract.

It was observed that by increasing the concentration of propolis extract, the antibacterial activity also increased. The extracts showed less antibacterial potential compared to standard antibiotic disc Gentamicin. The current study found that an ethanolic extract of Pakistani bee propolis had antibacterial potential against *Staphylococcus aureus*. Further research is needed to assess the biochemical contents of Pakistani bee propolis for use as a natural antibacterial agent in a variety of applications.

**Keywords:** Propolis, *Staphylococcus aureus*, *In vitro*.

#### Role of Probiotics in Prevention and Treatment of Colon Cancer

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#### **Abstract**

Cancer is one of the fatal conditions whose treatment is still in doubt. Various synthetic agents and (standard) chemotherapeutic drugs in treating cancer are not efficient. Researchers are trying to find competent clinical management for cancer as synthetic agents develop drug resistance, affect the quality of life, and are also not affordable to the patients. The purpose of this study is to highlight the importance of probiotics in preventing and treating cancer. Probiotics are actually living organisms that provide health benefits to the hosts (when administrated). Probiotics help control urogenital infection and diarrhea, alleviate lactose intolerance, reduce the level of cholesterol, and relieve the patients from bowel syndrome. Due to antioxidant potential, probiotics are beneficial for treating chronic diseases like cancer. The use of probiotics as vital dietary supplements is efficient in managing the side effects of radiation therapy, chemotherapy, and surgery. Proper administration of probiotics can reduce the inflammation of intestinal mucosa as they rebalance the gut microbiota and modulate the immunity of the gastrointestinal tract. Probiotics metabolome interferes in the progression of colon cancer. Short-lived metabolites (from milk) after getting fermented with Streptococcus thermophilus and Lactobacillus bulgaricus strains have efficient role in neutralizing the risk factors of colon carcinogenesis. Due to the ingestion of probiotics, those components that are genotoxic to the colon are excreted in small concentrations from urine, and those components that encourage oxidized DNA bases are excreted in high concentrations. Therefore, research on the anti-carcinogenic property along with the mechanism of action (particularly in treatment) of specific probiotics is desirable and important. Clinical trials should be conducted for the approval of probiotics validation as an alternative to colon cancer therapy from the medical community.

Keywords: Probiotics, Colon cancer, Lactic acid bacteria, Colon cancer risk, Gut microbiota

## Milk Borne Toxoplasmosis: Zoonotic Significance and Public Health Concern

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#### **Abstract**

Toxoplasmosis is a disease of zoonotic significance. Toxoplasmosis in humans might happen vertically by passing the tachyzoites to the embryo across the placenta or horizontally by consumption of foods adulterated by tachyzoites and bradyzoites, unpasteurized milk, cheddar, and raw meat. As a livestock by-product, milk is collected from dairy farms and supplied through Gowallas in unhygienic manners. *T. gondii* is an opportunistic agent for patients with HIV that can damage the heart, lungs, bone marrow, vision and cause potentially fatal encephalitis in these patients. The protozoon, *T. gondii* is an obligatory intracellular parasite that can cause disorders like abortion, the birth of deformed fetuses, mummification of the fetus, weakness of new young ones, embryonic reabsorption, and other reproductive diseases in small ruminants. Worldwide, the causative agent *T. gondii* has been identified in raw milk of sheep, goat, and camel by using various approaches, i.e., latex test, direct agglutination, indirect immunofluorescence antibody test, and ELISA. The tachyzoite stage is responsible for the transmission of Toxoplasmosis infection to humans. It is also an apprehension for pregnant mothers because tachyzoites can migrate transplacentally and cause abnormalities in human embryos.

Pakistan lacks a modern agricultural system like many other countries, which increases the prevalence of *T. gondii*. After being transported to the slaughterhouse, meat is easily contaminated with *T. gondii*. Open markets are common in Pakistan and are a cause of pathogen contamination because stray cats, mice, etc., are commonly found in the markets. There are comparatively fewer reports on the seroprevalence of *T. gondii* in distinct parts of Punjab, Pakistan. Therefore, there is a dire need to plan an epidemiological investigation in various peri-urban areas of Pakistan. The quantified prevalence of toxoplasmosis and associated risk factors will help the stakeholders, i.e., farmers, public health officials, and policymakers devise appropriate control measures against this zoonotic disease.

Keywords: Toxoplasmosis, Raw milk, sheep, goat, camel, Serodiagnosis, Zoonosis

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#### Leishmaniasis: A Serious Public Health Concern in Pakistan

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#### **Abstract**

Leishmaniasis is a major public health concern, owing to both environmental and individual risk factors, such as large migrations, urbanization, deforestation, and new irrigation schemes. It is a disease of tropical and subtropical regions of Asia, Africa, America, and the Mediterranean. The causative agent Leishmania is transmitted in humans through the bite of sandfly (Phlebotomus and Lutzomyia species). The disease is ranked among the top seven deadly diseases of public health significance by the World Health Organization (WHO). Every year, 1.5 to 2 million new cases are reported worldwide; 350 million people are at risk of contracting the disease, and 70,000 people die from leishmaniasis. Clinical symptoms encompass a wide spectrum of signs with varying degrees of severity depending on the Leishmania species involved and the immune status of the host. The symptoms range from localized cutaneous to visceral and potentially lethal consequences. The disease manifests itself in a variety of ways, ranging from self-limited and even self-healing cutaneous manifestations to severe systemic disease, i.e., spleen and liver, etc. Lesions of leishmaniasis can appear everywhere on the body; however, the exposed areas are prone to attack. Although the organism that causes leishmaniasis was discovered 100 years ago, the disease has not been eradicated, and in many parts of the world, it is on the rise. It has the potential to become a severe health issue if no control measures are taken. The cutaneous form of leishmaniasis is common in various regions of Pakistan, including South Punjab, KPK, Baluchistan, Sindh, and tribal areas. It is the second most prevalent vector-borne disease in Pakistan after malaria. Several factors such as climatic and environmental changes, the movement or migration of infected people, animal reservoirs, and female infected sandflies play an important role in the transmission of leishmaniasis. Keeping in view the significance of this public health threat, studies should be carried out in the endemic areas of Pakistan. Reservoir hosts, humans, and other risk factors responsible for the transmission of disease in vectors and hosts must be quantified with the objective of informing policymakers to make evidence-based control strategies to combat this disease.

Keywords: Leishmaniasis, Vector-borne disease, Sandfly, Zoonosis, Reservoir hosts

# Can Thymoquinone and Zinc (Active Constituents of *Nigella Sativa*) Be Used Against COVID-19?

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#### Abstract

The COVID-19 pandemic emerged in China in December 2019. Caused by a pathogen called SARS-CoV-2, COVID-19 has infected millions of people worldwide. The SARS-CoV-2 causative agent of COVID-19 belongs to beta coronaviruses. Due to the fast spread and increasing mortality rate, cost-effective and internationally available COVID-19 vaccines with maximum therapeutic effects are under clinical trials. This review article will therefore demonstrate the potential impacts of Thymoquinone and Zinc (active constituents of *Nigella sativa*) as a therapeutic and preventive tool against SARS-CoV-2. Active components of *Nigella sativa* (common name: black seed) have shown historical significance as an effective herbal remedy that preserves balanced inflammatory response through chronic inflammation suppression as well as upgrading host immune response.

Thymoquinone (volatile oil) is the main component of N.sativa. It possesses anti-inflammatory, hepato-protective and anti-oxidant characteristics. Molecular docking studies proposed that thymoquinone suppresses SARS-CoV-2 by interfering with ACE2 receptors. HSPA5 (Heat Shock Protein A5) was considered to be a possible path for SARS-CoV-2 attachment and entry. Thymoquinone has a binding affinity with 6LU7 (protein of SARS-CoV-2), ACE2 and HSPA5. In this way, thymoquinone blocks the viral entry into the host cell.

Against any infectious agent like bacteria and viruses, zinc is systematically concerned with cell -mediated immunity. Coronaviruses belong to the Positive-stranded RNA (+RNA) viruses. Such viruses require RNA-dependent RNA polymerase (RdRp) enzymes for their replication process. Zn<sup>2+</sup> directly impairs nidovirus RNA synthesis. Another core enzyme for viral replication and assembly of functional viral proteins is SARS-CoV papain-like protease 2; the activity of this enzyme is also inhibited by Zn. For the treatment of COVID-19, zinc-chelating agents such as citrate and ethylenediaminetetraacetic acid (EDTA) alone or in combination are expected. Persons suffering from low levels of Zn are more susceptible to viral infections.

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