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Comparing antimicrobial action of paracetamol to different potencies of baptisia tinctoria and belladonna

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Abstract
Scientists currently debate the future of nanotechnology may be able to create many new materials and devices with vast range of application such as in nano medicine. The potency of homoeopathic medications increases with potentization. The thrown challenge to scientific community at large with the good clinical results. As we found that the homoeopathic medicine Baptisia tinctoria and Belladonna had shown good results on fever. According to calculation using avogadro's limit, preparations above 12C dilutions should have no source materials present. Even though 1M, 30C were above the Avogadro's limit they showed the presence of photochemical like glycosides**,** proteins and also showed good antimicrobial activity.

Keywords: paracetamol, potencies, baptisia, belladonna, antimicrobial

Introduction
Paracetamol is used worldwide for its analgesic and antipyretic actions [1]. It has a spectrum of action similar to that of NSAID's and resembles particularly the COX-2 selective inhibitors [2, 3]. Paracetamol is on an average, a weaker analgesic than NSAID's but often preferred because of its better tolerence. There is a considerable debate about the hepatotoxicity of therapeutic doses of paracetamol [4, 5, 6]. Much of the toxicity may result from overuse of combinations of paracetamol with opioids which were widely used. Homoeopathy is based on the principle of similia, administering to the sick substance that cause similar symptom in healthy person. In order to minimize the possible aggravation that such treatment could cause on the symptom of both original disease, Samuel Hahnemann proposed the pharmaco technical method of dynamization or potentisation to reduce the primary effect of medications and develop the latent dynamic power [7, 8, 9]. Belladonna and Baptisia are the remedies that have shown marked results on the fever [10, 11, 12, 13, 14]. Some homoeopathic medicines [15, 16, 17, 18] such as belladonna has shown antibacterial action on Streptococcus Pyogens [19].

2. Methodology
2.1Collection of materials: Mother tincture, 200C, 30C, 1M, of Baptisia tinctoria and Belladonna collected from Dr Wilmar schwabe India Pvt ltd. Paracetamol was taken from local pharmacy.

2.2 Phytochemical analysis:
Belladonna Q, 30C, 200C, 1M and Baptisia Q, 30C, 200C, 1M was used for all studies. All the reagents were prepared according to standard protocols. A preliminary phytochemical study to determine the phytoconsituents present was under taken for extracts prepared and the homoeopathic formulations. The ethanol extract was considered suitable since the homoeopathic formulations were in ethanol medium.

Test for Flavioids
Alkaline reagent test: To the different sample solutions, a few drops of sodium hydroxide solution was added. Formation of intense yellow colour, which turned colourless after adding few drops of diluted hydrochloric acid, indicated the presence of flavioids.

Test for alkaloids
Mayer's test: 1ml of mayer's reagent was added to the different sample solutions. Formation of cream colour precipitate indicated the presence of alkaloids.

Test for glycosides: 1ml of samples was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of glycosides.

Test for saponins: 1ml of sample with 20ml of water was taken in a flask and shaken vigorously formation of stable froth for at least 10min indicates the presence of saponins.

Test for Amino acids
Ninhydrin test: A solution of ninhydrin ethanol was added to the added to the sample solution appearance of purple color indicated the presence of amino acids.

Test for Tannins: Take 2-3ml of sample and add 5% Fecl3 solution. The deep colour indicated the presence of tannins.

Test for phenols
Ferric chloride test: The sample was treated with 3-4 drops of ferric chloride solution. The bluish black colour indicated the presence of phenols.

Test for proteins
Xantho proteic test: The sample was treated with few drops of concentrated nitric acid, than the formation of yellow color indicated the presence of proteins.

Test for steroids and tarpenoids
One ml of sample and one ml of chloroform was added and 2-3 ml of acetic anhydride was mixed then 1-2 drops of concentrated sulphuric acid was added. Then the dark green colouration of the solution indicated the presence of steroids and pink or red colouration indicated the presence of terpenoids.

Test for carbohydrates
Benedict's test-The samples were treated with Benedict's reagent and heated gently. Orange ppt indicated the presence of reducing sugar

2.3Antimicrobial activity
The samples were collected from Alva's biotechnology research unit and done for all the medicine samples with keeping amoxicillin as positive control for antimicrobial activity. Paracetamol is taken along with the homoeopathic medicines to compare the actions.

Preparation of plates
1. A sterile cotton tipped swab was dipped into one of the broth cultures, and was used to inoculate a Mueller-Hinton agar plate using the procedure. Inoculation of the plate in this way ensures a lawn of bacterial growth after incubation. This inoculation procedure was repeated for a second plate using the same organism label both plates.
2. Step 1 was repeated for other 3 cultures which had a total of 8 inoculated and labelled plates, two for each culture. After inoculation, all plates were allowed to dry for 15 minutes before proceeding to next step.
3. 70% ethanol was poured into a 250ml beaker.
   - Forceps were dipped into the alcohol, and then forceps were passed over the Bunsen burner flame to sterilise them.
   - The antibiotic disc from one of the petri dishes, were placed one on the inoculated plates.
   - After placement on the agar, it was tapped once to make sure it is secure.

   The steps were repeated to see until you have placed this disc on a plate for each culture. Then it was proceeded to the next disc until five discs were placed on a plate for each culture. The other five discs were placed on the second plate, for a total of 10 discs per culture.
4. Then all the discs were placed, and 8 plates were placed into 35 ^{o}C incubator.

Examination of plates
1. The plates were examined after 16-18 hours of incubation.
2. Examination of plates was done for zones of inhibition. These places were measured with a millimeter ruler across the disc. The diameter of the zone was recorded to the nearest whole mille metre. If only one side of the zone could be measured, then multiply the number obtained by 2 to obtain a full zone of inhibition. If there was no zone (i.e, if growth occurs up to the edge of the disc), then it was recorded record a zero.

   Note: you might see colonies within the zone of inhibition. These colonies consist of cells that were resistant to the antibiotic. Continue recording the zones of inhibition until you have all 40 measurements.
3. Comparision was made of zone of inhibition obtained to the interpreting standards of these antibiotics. It was recorded whether each organism is resistant, susceptible, or intermediate to the antibiotic.
4. This exercise was completed by recording for each type of bacteria the antibiotics they were susceptible too. These represent possible drugs of choice to treat infections by these bacteria.

SEM
Scanning electron microscopy (SEM) is a test process that scans a sample with an electron beam to produce a magnified image for analysis. The method is also known as SEM analysis and SEM microscopy, and is used very effectively in micro analysis and failure analysis of solid inorganic material. Electron microscopy is performed at high magnifications, generates high resolution images and precisely measures very small features and objects.
Scanning electron microscopy uses a focused beam of high energy electron to generate a variety of signal at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected werea of the surface of the sample and a two dimensional image is generated that displays spatial variations in properties including chemical characterisation, texture and orientation of materials. The SEM is also capable of performing analyses of selected point location on the sample. This approach is especially useful in qualitatively or semi qualitatively determining chemical compositions, crystalline structure and crystalline orientations.
The EDS detector separates the characteristic x-rays of different elements into an energy spectrum and EDS system

software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrait as a plot of x-rays counts vs energy (in kev). Energy peaks corresponds to the various elements in the sample. Energy despresive x-ray spectroscopy can be used to find the chemical composition of materials down to a spots size of a few microns and to create element composition maps over a much broader raster were together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers and metals.

And result was compared with the SEM of paracetamol.

FTIR

Fourier transform infrared spectroscopy was the technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas.

The medicinal samples were undertaken for FTIR to identify the organic, polymeric, and in some cases inorganic materials and the graph was compared with the FTIR of the paracetamol.

Results and Discussion

Phytochemical analysis-

Table 1: xxxxx

Sl No	Phyto consituents	Bell Q	Bell 1M	Bell 200	Bell 30	Bapt Q	Bapt 1M	Bapt 200	Bapt30
1	Flavinoids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Glycosides	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
4	Saponins	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
5	Tarpenoids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Tannins	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Phenol	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
8	Protien	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
9	Carbohydrates	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

Fig 1: Carbohydrates,

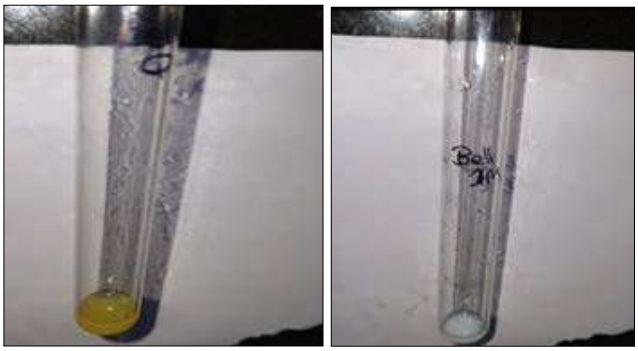

Fig 2: Glycosides A; bell Q B; bell 1M

Fig 3: Glycosides- A; bapt Q B; bapt 1M

Fig 4: Saponins; bapt

Fig 5: Saponins; Bell,

Fig 6: Alkaloids A; bapt Q B; bell Q

Fig 7: Tannins

Fig 8: Proteins

SEM

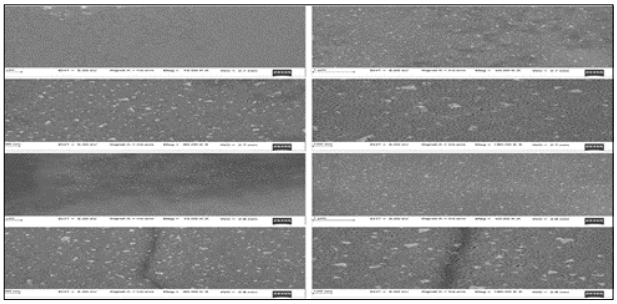

Fig 1: BellQ

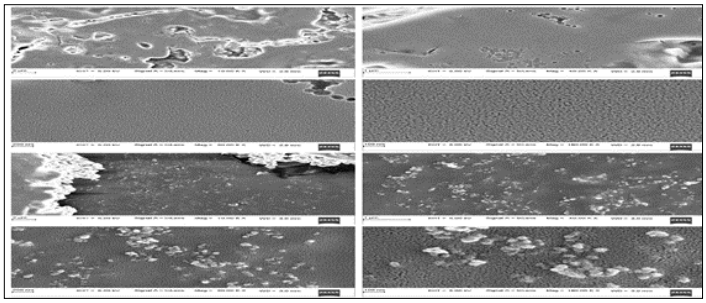

Fig 2: Bapt Q

FTIR

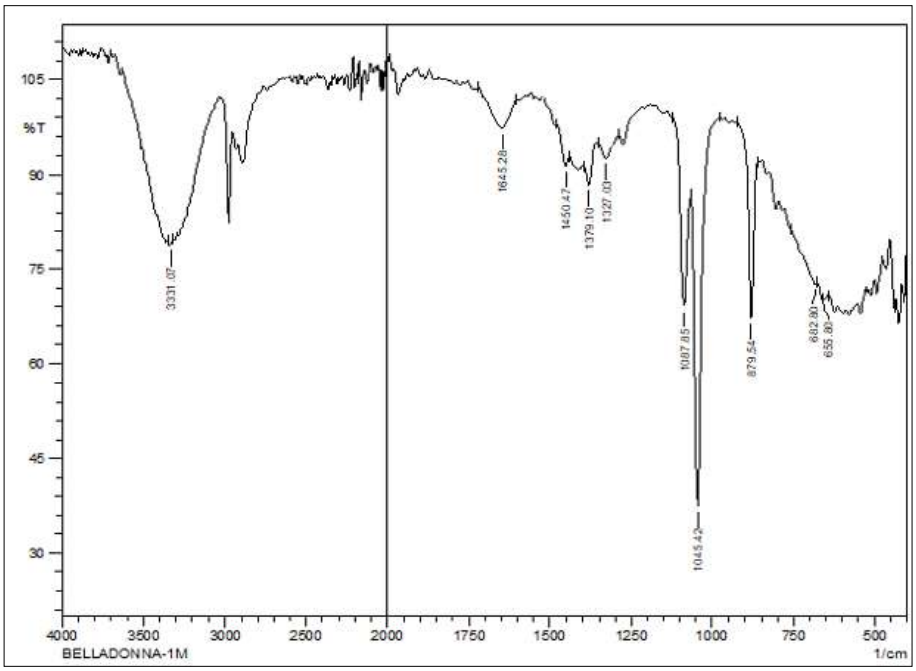

Fig 1: bell1M.

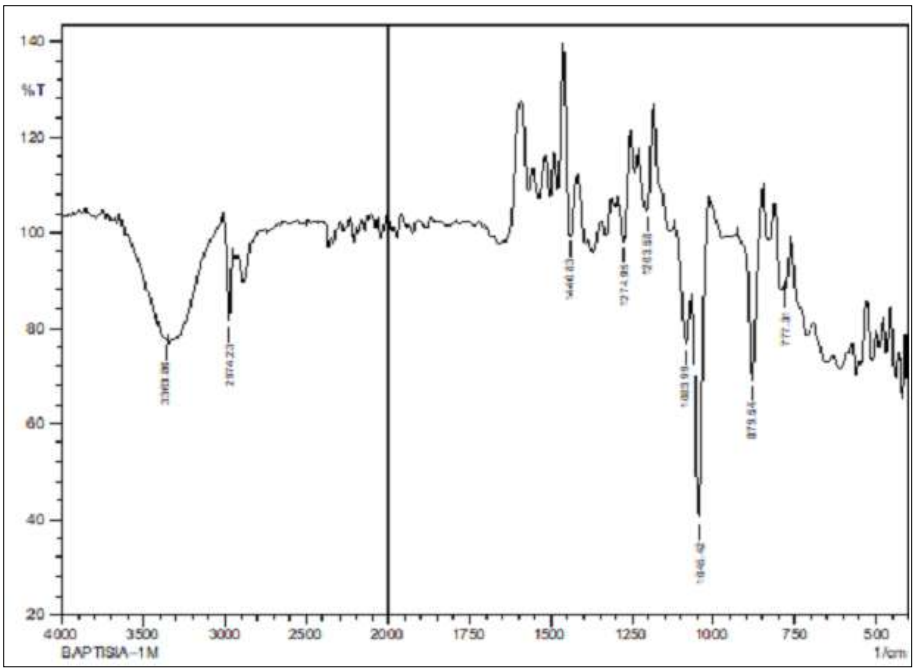

Fig 2: BellQ.

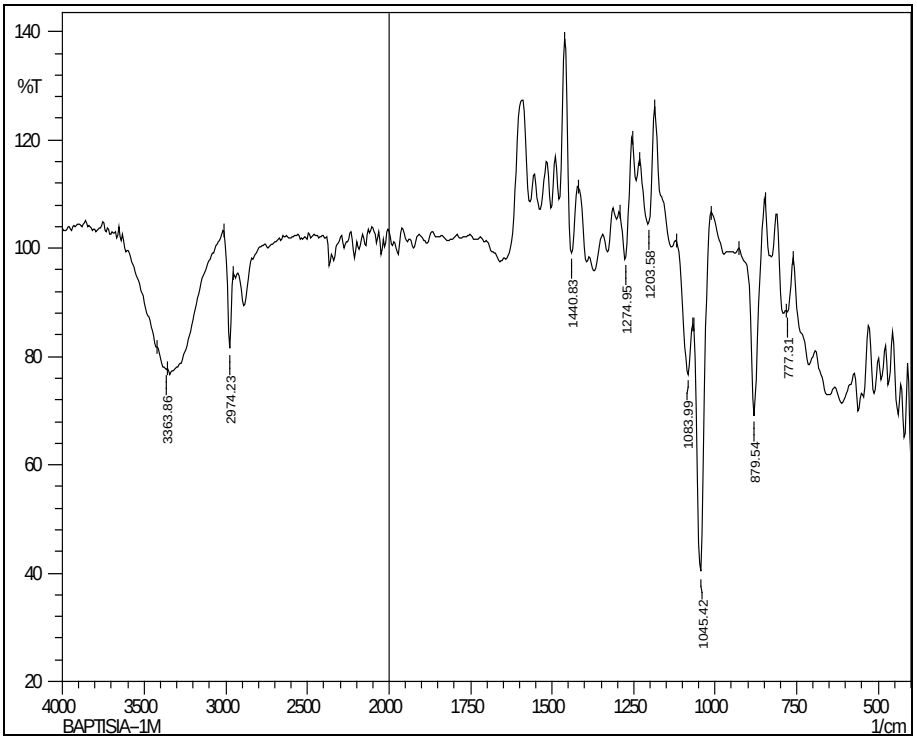

Fig 3: Bapt1M

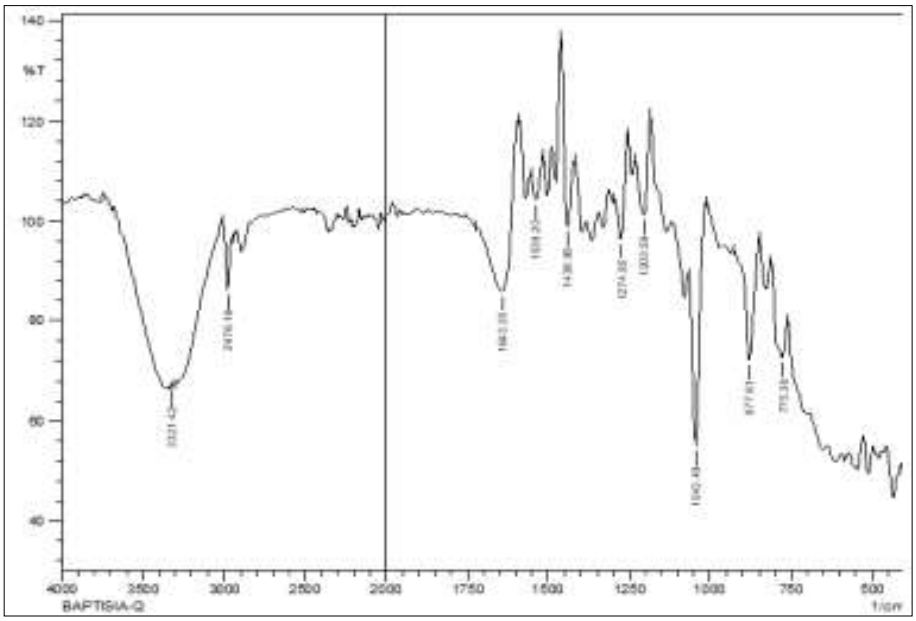

Fig 4: Bapt Q

Conclusion

The Paracetamol has mild antimicrobial activity which increases linearly with concentration of but it harms the gut flora if given in high dose [20]. Baptisia tinctoria and Belladonna had good clinical results on fever. Even though 1M and 30C are above the Avogadro's limit, they showed the presence of phytochemical like glycosides, proteins and also showed good antimicrobial activity. So scientifically there is value for Baptisia tinctoria and Belladonna in treating pyrexia. Further study with different microbes and with the HPLc may help to make it more scientific.

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