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Echinacea purpurea Extract Affects the Immune System, Global Metabolome, and Gut Microbiome in Wistar Rats

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Abstract

Echinacea purpurea extract, a traditional herbal food additive with dual-purpose of medicine and edible material, has been widely used for the treatment and prevention of various infectious diseases, especially for children, old aged and immunocompromised patients. Although there were numerous reports suggested *E. purpurea* possessed immunostimulatory and antibacterial effects *in vitro*, the mechanisms underlying remained to be elucidated. This study employed immunologic factors analysis, GC-TOFMS based metabolomics and 16S-rRNA-sequencing microbiome profiling technologies to explore the effects of *E. purpurea* on young rats, a physiological insufficient immunity status, by compared with pidotimod treatment on young rats and adult animals. *E. purpurea* treatment significantly increased IL-2, decreased IL-6 and affect immunoglobulins in the spleen of young rats, indicating its promotion of cellular immunity. Both the immunologic factors and the global metabolome of *E. purpurea* treated young rats were close to the status of mature individuals. Results of 16S-rRNA-sequencing of ileum content together with co-metabolism metabolites demonstrated that *E. purpurea* changed gut microbiota structure characteristically as a reducing Firmicutes phylum, especially *Lactobacillus*, and a rising *Actinobacteria* phylum including *Bifidobacterium*. The results were concluded that *E. purpurea* could potentially promote the maturation of immune and metabolism of immature rats, and also affect gut flora structure.

Keywords: *Echinacea purpurea* extract, metabolomics, immunomodulation, gut microbiota

1. Introduction

Echinacea purpurea Moench (*E. purpurea*) is an indigenous medicine of the native American Indians and Europeans with multiple biological activities, such as anti-inflammation, anti-oxidation, anti-bacteria, antiproliferative, antihypertensive and immunomodulation effects for hundreds of years (Aarland et al., 2017; Chiou et al., 2017; LaLone et al., 2007). This medicinal plant was also used as food supplements in infectious diseases in children, old people and animals in North America and Europe (Ayrle et al., 2016). As a famous

alternative therapeutic herb, *E. purpurea* is usually considered to be efficient and natural with low toxic and fewer side effects. It was also mixed in the fodder as food supplements in animal husbandry (Chen et al., 2014). The major ingredients of *E. purpurea* are choric acid, caffeic acid, and other polyphenols and glycoproteins. Glycoproteins, alkylamides and polysaccharides in roots of *E. purpurea* chemical compounds are believed main responsible for their immunomodulatory properties (Balciunaite et al., 2015). It has been reported that the activation of immune cells exhibited by *E. purpurea* arose from the presence of the bacterial components (Nirmal, 2008). The immunomodulatory effect of *E. purpurea* was reported to involve biochemical alterations in immune cells and intestinal bacteria, but the exact underlying immunomodulation mechanisms involving plant metabolites and microbiome are yet to be understood (Nirmal, 2008; Todd et al., 2015).

Accordingly, the metabolic profiling associated with 16S rRNA sequencing and conventional immune factors was used in this study to discover the effects of *E. purpurea* on systemic immune, metabolome and gut microbiome on young rats after weaning *in vivo*. The experiment was designed to compare the actions of *E. purpurea* with adult rats, and pidotimod (3-l-pyroglutamyl-l-thiazolidine-4 carboxylate), a synthetic dipeptide immunomodulatory, to explore the potential effects of *E. purpurea* on immature rats.

2. Materials and Methods

2.1 Animal Experiments

Wistar rats were born by the mother rats purchased from Shanghai Laboratory Animal Co Ltd. (SLAC, Shanghai, China). After-born, both male and female rats were raised separately. All experimental rats(male rats, 10 rats each group) were housed in a clean environment under a controlled condition of 12 h light/12 h dark cycle at 20-22 °C, and the humidity of the environment was 45 ± 5%, with free access to sterilized chow and water. At age of 3 weeks, the rats were randomly divided into four groups: the normal group (N), the *E. purpurea* group (EE), the pidotimod group (P) and the adult group (14w). N group and 14w group were fed as before. The rats in EE group were oral administration of *E. purpurea* (bought from Xi'an Xiaocao Botianical Development Co. Ltd., polyphenol 4%) at a dose of 600 mg/kg dissolved in the water. And the rats in P group were oral administration of pidotimod (bought from Sunstone Co. Ltd.) at a dose of 150 mg/kg dissolved in the water. The water was changed every day. The N, EE and P groups were killed after 6 weeks while the 14w group was killed after 11 weeks. The day before their death, the urine samples were collected in the metabolic cages for acclimatization. The urine samples collected should be centrifuged at speed of 3000 rpm for 10 min and the supernatant was subsequently moved to another eppendorf tube before putting into the -80 °C refrigerator. All of the rats above were sacrificed to collect whole spleens, small intestinal contents and serum samples. All of the samples were stored separately at -80 °C before analysis.

2.2 Measurement of Immune Factors in Spleen

The level of immune factors, immunoglobulins and NK cell in the spleen of 6 rats in each group were measured by an ELISA kit (IL-2, IL-4, IL-6, IL-10, IgA, IgM, IgG and NK cell ELISA Kit, Shanghai Jianglai Biotech, Shanghai, China). All steps followed the manufacturer's instructions.

2.3 Sample Pretreatment and GC/TOFMS Analysis

Serum and urine samples above were pretreated according to previously published methods (Hou et al., 2015; Zhang et al., 2016). To minimize systematic analytical deviations, the samples were run in the order of "N group-EE group-P group-14w group" alternately. Separation was achieved on a DB-5ms capillary column (30 m × 250 μm i.d., 0.25 μm film thickness; (5%-phenyl)-methylpolysiloxane bonded and cross-linked; Agilent J&W Scientific, Folsom, CA), with helium as the carrier gas at a constant flow rate of 1.0 mL/min.

2.4 DNA Extraction and 16S-rRNA-Sequencing-Based Studies of the Ileum Microbiota

The ileum contents samples of 5 rats in each group were removed from storage. DNA was extracted using the QIAamp Stool Mini kit (Qiagen). The V4 region of the 16S ribosomal RNA (rRNA) gene from DNA samples was amplified using the bacterial forward primer 5'-AYTGGGYDTAAAGNG-3' and the reverse primer 5'-TACNVGGGTATCTAATCC-3'. PCR amplification, pyrosequencing of the PCR amplicons, and quality control of raw data were performed, as described previously (Zhang et al., 2010).

2.5 Statistical Analysis

The acquired GC/TOFMS data were processed which included smoothing, peak picking, denoising, identification, and alignment using ChromaTOF software (v4.22; Leco Co.) as described in a previous publication (Cheng et al., 2012). Compounds information, peak retention time and peak area were all included in the final dataset. The final peak areas were normalized to internal standards before statistical analysis. The

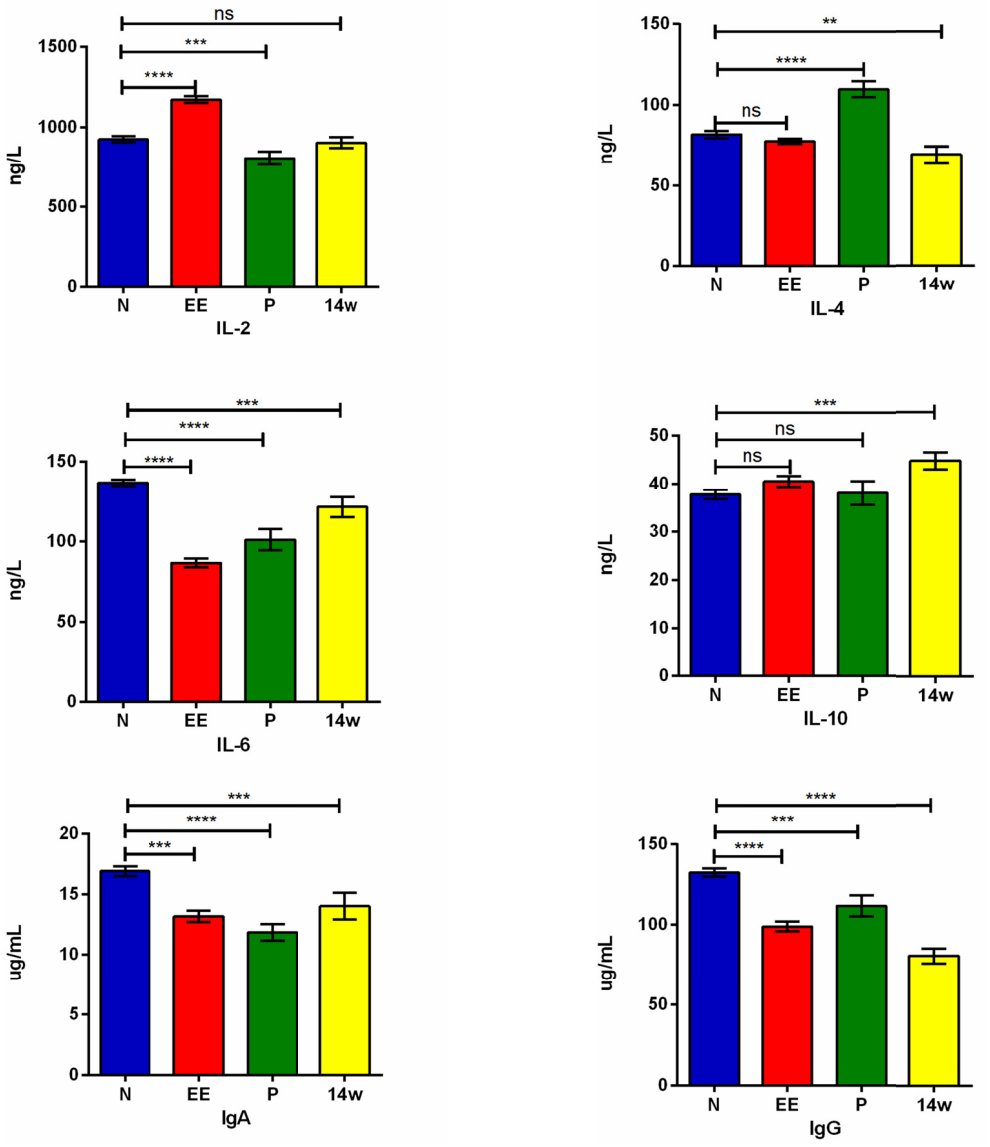
principal component analysis (PCA) was performed with SIMCA-P (v 13.0; Umetrics) software. Differences and differentiating variables selection were considered statistically significant at a Student *t* test ($P < 0.05$) and Fold Changes ($FC > 1.2$ or $FC < 0.8$). Statistical comparisons included paired t test and other classic physiological indices in rats during the process of different administration groups. *GraphPad Prism* version 5.0 software program (San Diego, CA) and *galaxy* (https://huttenhower.sph.harvard.edu/galaxy/) was used in statistical analysis.

For the sequencing data, we used QIIME to separate the sequences into different (Operational Taxonomic Units, OTUs) according to the similarity of the sequences. Using the RDP database and RDP-classifier as training set to note the OTUs (Maidak et al., 1997).

3. Results

3.1 Immune Indexes in the Four Groups

From the result of Elisa analysis, it could be found IL-2 in the spleen increased after taking *E. purpurea* extract (Figure 1). The contents of IL-6, IgA, IgM, IgG and NK cell were decreased significantly by EE, while IL-4 and IL-10 didn't change. Results of 14w group (14 weeks) were significantly different from that of N group (9 weeks), indicating age was an important factor affecting the immune system.



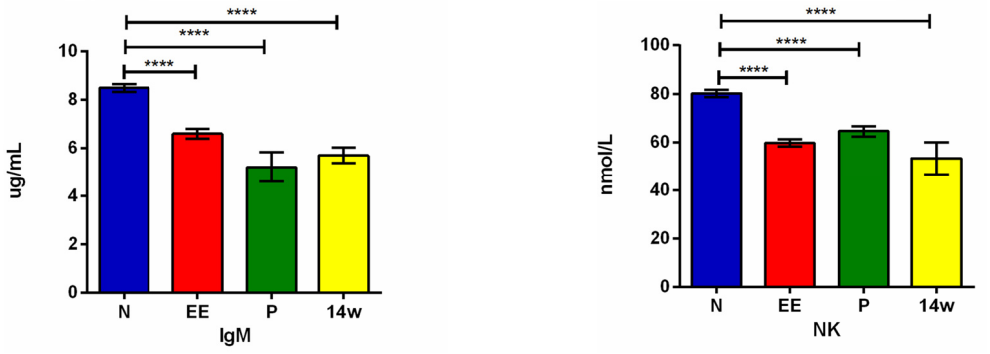
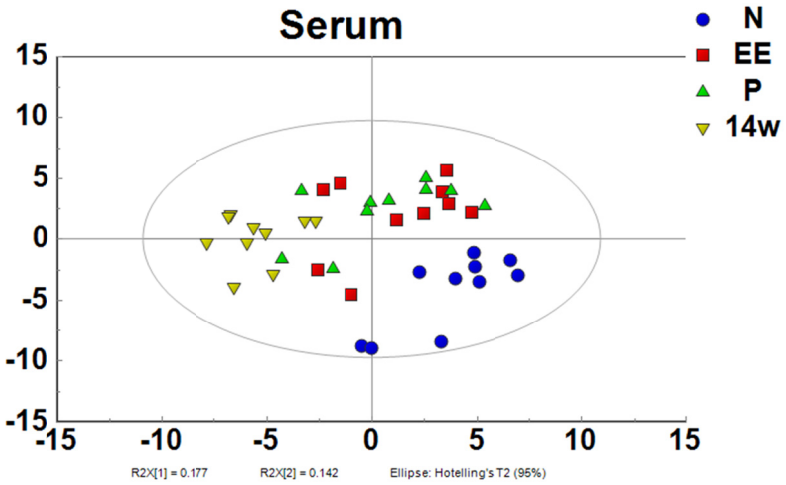


Figure 1. Immune factors, immune globulin and NK cell contents in spleen by ELISA kit

Note. Values shown were means +/- SD from a single representative experiment (n = 6). Statistical analysis was performed using the Student's T test, *p < 0.05, **p < 0.01, ***p < 0.001, **** < 0.0001.

3.2 Metabolic Profiling of Serum and Urine in the Four Groups

A total of 39 metabolites associated with amino acid metabolism, tricarboxylic acids (TCA) cycle, carbohydrate metabolism, fatty acid metabolism and lipid metabolism were altered by EE in the serum. 27 out of 39 changed metabolites (EE/N) were in the same direction between that of EE and 14w groups, while out of 31 changed metabolites (EE/N) were in the same direction between EE and P groups. The PCA scores plot derived from GC/TOFMS data was depicted in Figure 2A. It showed there was a clear separation observed in the plot of serum samples among N, EE and 14w groups. EE and P groups had the same tendency with the 14w group (Figure 2B). From the heatmap, most amino acids were up-regulated by EE, except glutamine, pyroglutamate and N-formyl-L-glutamate. Most sugars and polyols were down-regulated in all three groups as compared to N group.



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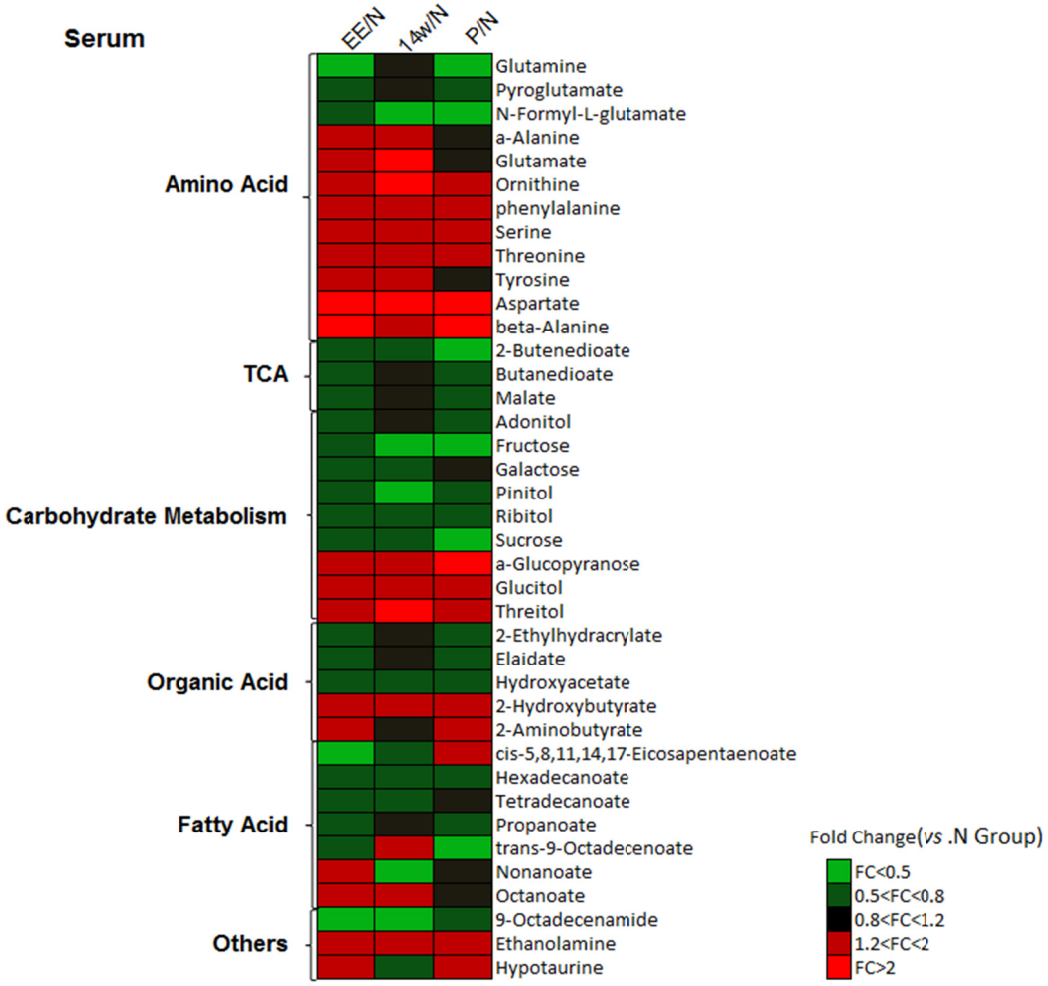

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Figure 2. Metabolic variation in rat serum (n = 10). A: PCA scores plot among the N, EE, P and 14w groups; B: heatmap of the serum metabolites. EE/N means the Fold Change of EE Vs N group

Note. 14w/N means the Fold Change of 14w Vs N group. P/N means the Fold Change of P Vs N group. All of the outcomes were normalized by their total peaks.

Urine metabolites were more complicated than serum metabolites (Table 1). PCA scores scatter plot can't show satisfactory discrimination among the four groups. However, PLS-DA scores scatter plot (Figure 3) was performed on the integrated MS data to identify the metabolites that contribute the classification of samples and also to obtain better discrimination between the treatment and normal groups in our study.

Table 1. Most altered metabolites and their changes in serum and urine (n = 10)

Serum	FC (EE/N)	Pvalue (EE/N)	FC (14 w/N)	Pvalue (14w/N)	FC (P/N)	Pvalue (P/N)
Glutamine	0.482472	0.047547	1.075944	0.357815	0.42038	0.012609
Pyroglutamate	0.618208	0.031565	1.129382	0.124377	0.768354	0.006477
N-Formyl-L-glutamate	0.624332	0.004816	0.36664	0.00404	0.452618	0.21981
phenylalanine	1.350962	0.011806	1.5378	1E-06	1.277466	0.002618
Glutamate	1.376729	0.048742	2.042893	8.21E-07	1.166887	0.02524
Tyrosine	1.411447	0.048761	1.688152	7.74E-06	1.023062	0.000683
Serine	1.426597	0.000513	1.63138	1.7E-06	1.366247	0.018667

Threonine	1.447236	2.1E-06	1.447681	1.02E-06	1.408453	0.000367
a-Alanine	1.455853	0.048996	1.525206	5.06E-05	1.015877	0.00881
Ornithine	1.531911	0.038894	2.609411	5.26E-08	1.478018	0.11376
Aspartate	2.469403	3.36E-06	2.691249	5.67E-12	2.15994	4.26E-06
beta-Alanine	2.531269	3.21E-05	1.514046	0.016346	2.595981	1.45E-05
2-Butenedioate	0.612522	0.017211	0.730709	0.330421	0.495211	0.001958
Butanedioate	0.675581	0.031717	1.055729	0.227592	0.581517	0.020435
Malate	0.759408	0.016945	0.866559	0.268401	0.645026	0.022669
Adonitol	0.763162	0.046659	1.034162	0.299437	0.78851	0.029303
a-Glucopyranose	1.986988	0.001265	1.678212	0.015034	2.371897	0.01042
Fructose	0.535557	6.68E-08	0.450993	7.71E-08	0.462019	3.4E-07
Galactose	0.732033	0.032229	0.780963	0.105601	0.894637	0.079656
Glucitol	1.602747	0.00109	1.653818	3.35E-06	1.481416	7.45E-05
Pinitol	0.548279	4.46E-05	0.397404	7.8E-09	0.616687	0.000216
Ribitol	0.52243	2.83E-08	0.635726	2.31E-08	0.55589	7.82E-10
Sucrose	0.633938	0.003796	0.556875	0.134673	0.486835	0.129236
Threitol	1.486085	0.003295	2.199111	1.72E-05	1.270938	1.25E-05
2-Ethylhydracrylate	0.79067	0.002382	1.094304	0.249812	0.751117	0.011142
2-Hydroxybutyrate	1.414603	0.047628	1.328419	0.047004	1.33695	0.030115
Elaidate	0.618278	0.001365	1.010583	0.082998	0.616846	0.022274
Hydroxyacetate	0.546022	0.005363	0.660644	0.009099	0.602782	0.006295
2-Aminobutyrate	1.671816	0.002153	1.032849	0.260327	1.429875	3.48E-05
Nonanoate	1.2423	0.016187	0.45638	0.005259	0.975025	0.455308
Octanoate	1.457768	0.005886	1.47156	0.030447	1.18409	0.033322
Propanoate	0.646445	0.00458	0.854677	0.001848	0.788766	1.16E-05
cis-5,8,11,14,17-Eicosapentaenoate	0.276607	0.024633	0.576539	3.63E-09	1.928515	0.012573
Hexadecanoate	0.572611	0.044106	0.660664	7.79E-06	0.678792	0.095875
Tetradecanoate	0.759902	1.63E-05	0.747952	2.86E-05	0.80777	9.0836E-05
trans-9-Octadecenoate	0.608076	0.000943	1.21415	0.202154	0.436346	0.00346
9-Octadecenamide	0.371867	0.017459	0.382553	0.000881	0.513681	0.040912
Ethanolamine	1.401043	0.00441	1.85272	3.42E-09	1.208676	0.001334
Hypotaurine	1.599124	0.004767	0.746888	0.2833	1.72439	0.015981
Benzoate	0.042014	0.003527	7.456914	0.004977	0.031509	0.003314
4-Hydroxycinnamate	0.177439	0.038439	3.812942	0.034499	0.183102	0.038773
Benzeneacetate	0.326647	0.003253	5.376051	0.008507	0.229609	0.001359
p-Cresol	0.358233	0.011335	3.750271	0.000113	0.204995	0.003524
Lactate	0.499429	0.007873	2.193151	0.048429	0.304838	0.001101
Glycine	0.563747	0.011059	2.27822	0.028177	0.324177	0.000671
Quinolinate	0.63656	0.004614	0.818062	0.13127	0.448338	0.000168
Proline	0.688347	0.03225	0.585473	0.011903	0.477745	0.00207
Hippurate	1.375942	0.049617	1.566047	0.109155	1.007415	0.482886
Hydrocinnamate	1.39555	0.024018	4.453012	0.00021	1.460509	0.031996
1H-Indole	2.069217	8.97E-05	1.484211	0.005453	1.447461	0.001801
Glycerol	2.075444	0.002349	0.343988	0.005923	1.801611	0.005983
2'-Deoxycytidine	2.107295	0.009614	0.429451	0.014141	2.200168	0.000183
Ornithine	2.246376	0.000147	0.867554	0.286783	1.727258	0.001758
Norleucine	2.294829	0.000876	0.473146	0.043601	1.694956	0.026174
Glucose	3.165048	0.003636	0.1145	0.044839	2.1365	0.023389
4-Pyrimidinamine	3.603864	0.010001	0.858872	0.401089	3.986408	0.000429
3-Methylazelate	3.631625	0.000207	6.362245	0.000187	2.590256	0.004074
Mannose	3.684116	0.00013	0.091526	0.037641	2.379516	0.007041

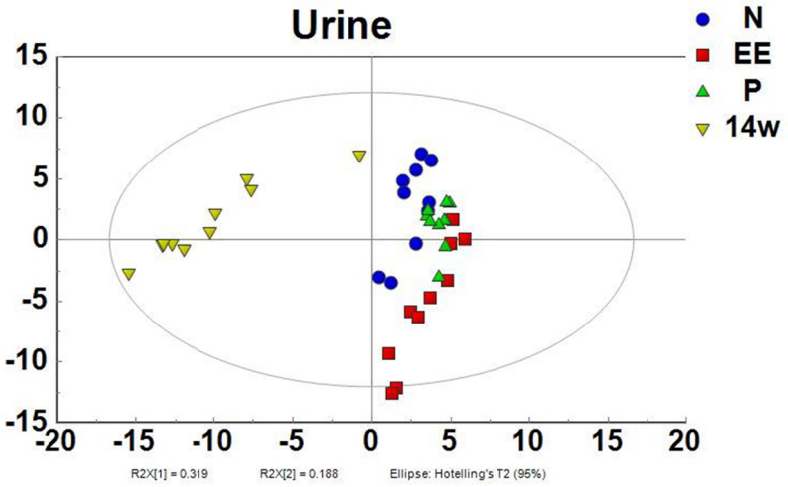


Figure 3. PLS-DA score plot among the N, EE, P and 14w groups in the urine metabolites

3.3 Overall Structural Changes of Gut Microbiota in the Four Groups

After drinking *E. purpurea* and pidotimod for six weeks, the bacterias in the ileum also changed a lot (Figures 4 and 5). First of all, the abundances of *E. purpurea* and pidotimod are larger than the normal group, and the structures of them are not the same. As compared with N group, Firmicutes (Figure 5B) were significantly decreased in EE group, Proteobacteria (Figure 5C), Actinobacteria (Figure 5A) and others were increased in EE group. *Bacillus*, *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Allobaculum* are the dominant genus in Firmciutes. In this phylum, Lactobacillus was decreased largely in the ileum, from 81.5258% decreased to 22.534% in abundance. Streptococcus was also decreased in the EE group, while *Bacillus*, *Lactococcus* and *Allobaculum* were increased in the EE group. In the phylum of Proteobacteria and Actinobacteria, all the dominant genus like *Pseudomonas*, *Rothia*, *Mycobacterium*, *Bifidobacterium* and *Acinetobacter* have increased along except *Aggregatibacter*.

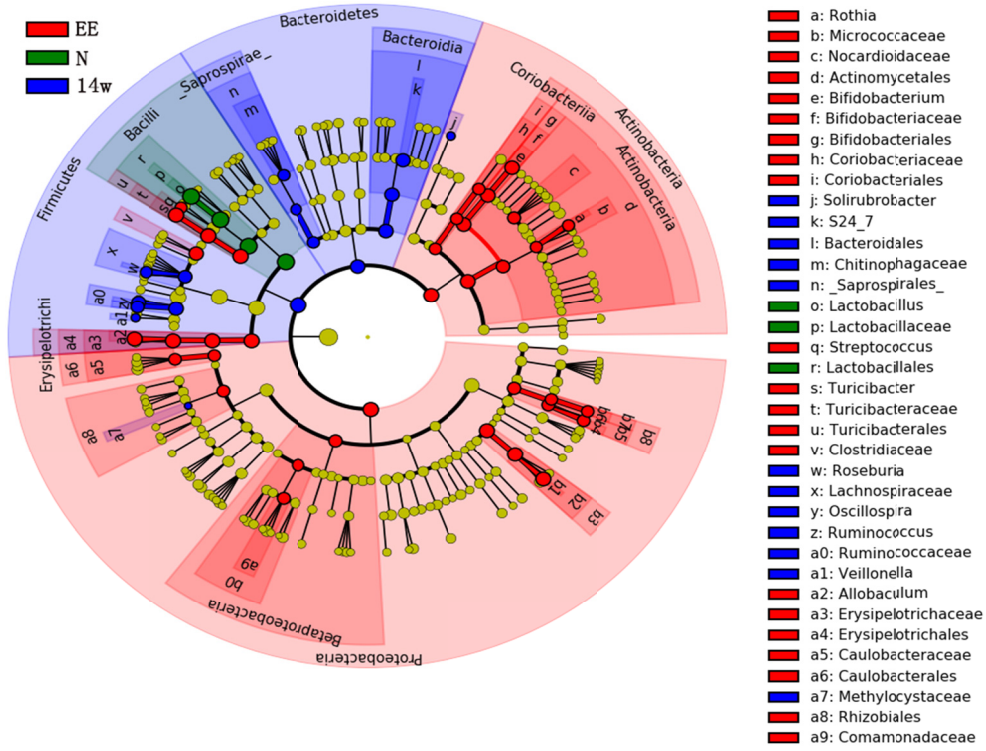
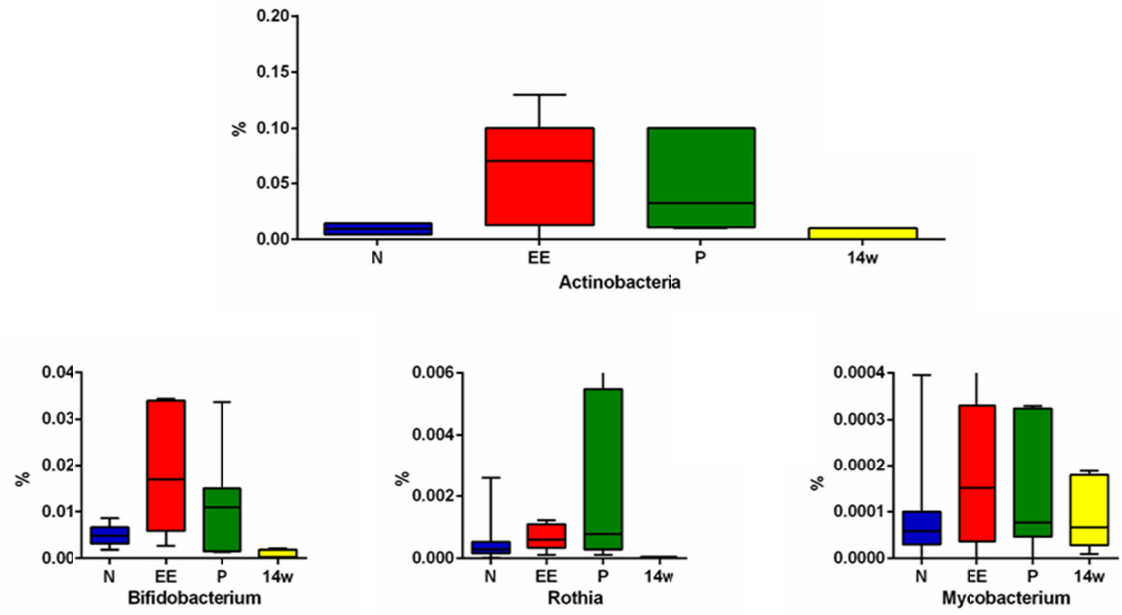


Figure 4. LEfSe analysis of gut microbiota (n = 5)

Note. The circles from the outside to inside indicate genus, family, order, class, phylum, and kingdom. Red indicates this taxon was more present in EE group; Green indicates this taxon was more present in N group; Blue indicates this taxon was more present in trial group than in 14w group.



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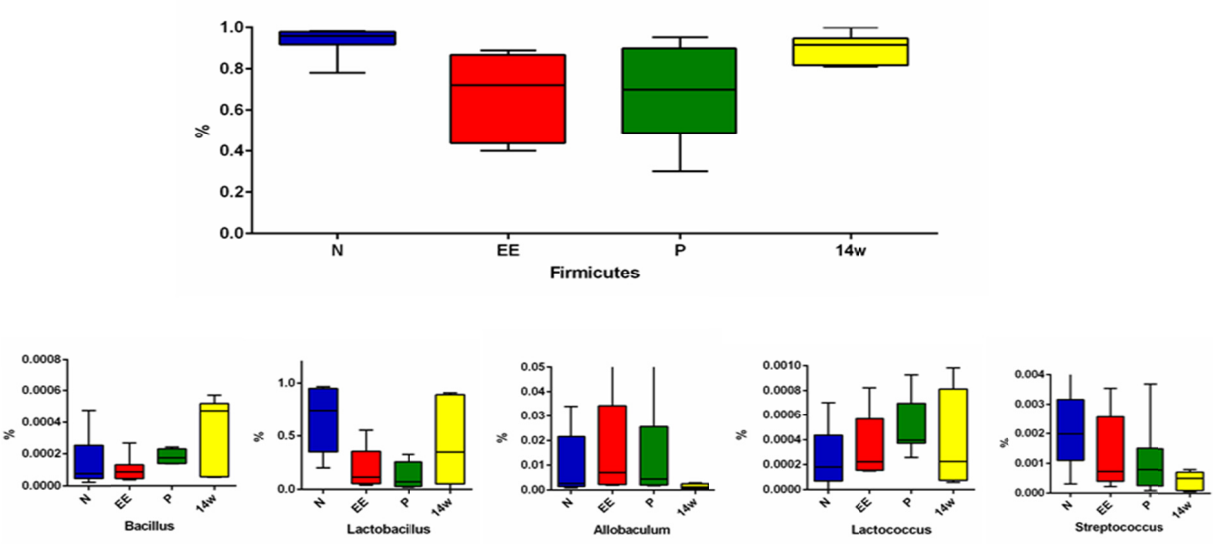

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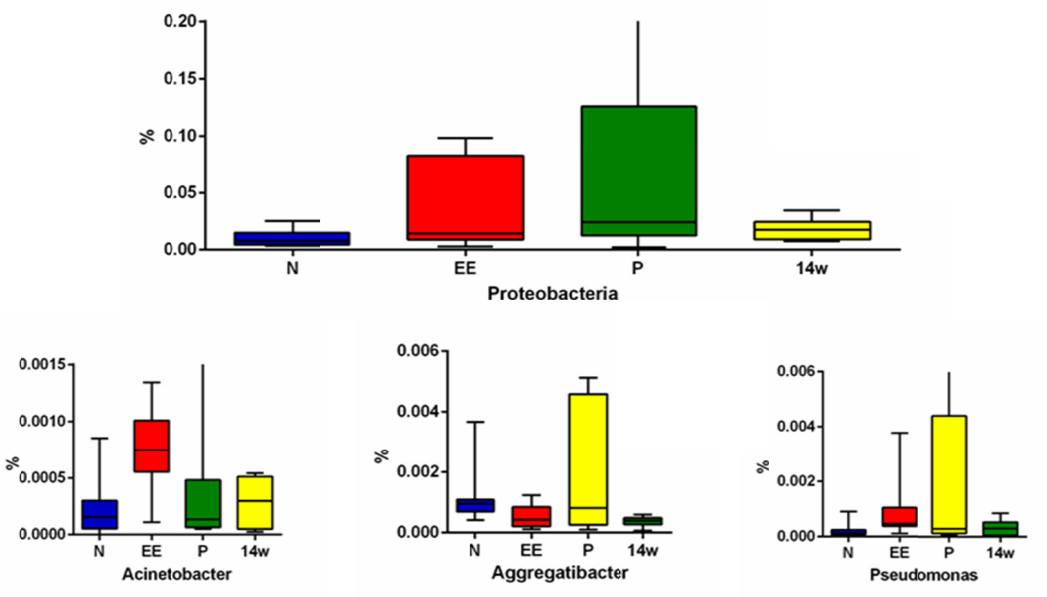

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Figure 5. The main changed phyla and genera of gut microbiota (n = 5)

Note. A: The relative abundance of Actinobacteria and its dominate genera. B: The relative abundance of Firmicutes and its dominate genera. C: The relative abundance of Proteobacteria and its dominate genera. The interquartile range is indicated by the outer bounds of the boxes, the median is indicated by the black midline, and the circles represent the outliers. The whiskers represent the minimum and maximum.

4. Discussion

By comparing the actions of *E. purpurea* with pidotimod on immunoregulatory cytokines, immunoglobulins, systemic metabolome and gut microbiome in immature rats, and then with this status in adult rats, our study revealed that *E. purpurea* and pidotimod had high similarity in regulating immunoregulatory cytokines, immunoglobulins and metabolites. Furthermore, *E. purpurea* made the body's metabolism close to the adult state.

4.1 The Effect of EE on Immunoregulatory Cytokines and Immunoglobulins

Pidotimod has been definitely confirmed to have immunomodulatory activity on both innate and adaptive responses via acting on different immunological pathways (Esposito et al., 2015; Mameli et al., 2015; Namazova-Baranova et al., 2014). Pidotimod is also believed a safe immune stimulating agent by stimulating nonspecific immunity, humoral immunity and cellular immunity, with no serious adverse event occurred and even being used as a child medication (Licari et al., 2014).

The observation in our research shows that *E. purpurea* and pidotimod both reduced IL-6, IgA, IgG, IgM and NK levels in spleen, presented the same tendency with that of 14 weeks' rats, suggesting *E. purpurea* might have a positive effect on immune system (Brousseau & Miller, 2005). Different from pidotimod, almost on the contrary, *E. purpurea* significantly increased IL-2. IL-2 is generated by Th1, and IL-6 is produced by Th2. So the regulation of *E. purpurea* on Th1 and Th2 is totally different (Mosmann & Coffman, 1989). It has been reported that *E. purpurea* directly affected the immune system such as T lymphocyte, macrophage, dendritic cell (Cech et al., 2010; King et al., 2014). IL-2 was proven to promote the proliferation of T cells and enhance the cellular immunity, associated with the production of IgM and IgG (Cozzi et al., 1995). IL-6 promotes the activation of B cells, and facilitates humoral immunity together with IL-4 and IL-10. IL-6 can also contribute to the exudation and chemotaxis of inflammatory cells, cause fever, participate in inflammation and pathological damage, and improve the super sensitive reaction together with IL-4 (Vazquez et al., 2015). Because IL-6 is a terminal differentiation factor for B lymphocytes and it can enhance body's Ig production, the decreasing IL-6 level in spleen was associated with the reduced level of IgA, IgG, IgM (Hirano et al., 1985). IgM is an immunoglobulin with largest molecular weight existing in circulation, and believed the main immune response in the primary immune response (Suzuki & Tomasi, 1979). In combination with the above results, it could be concluded that the effect of *E. purpurea* may exist in the promotion of cellular immunity, and the reduction of cell inflammation and the damage caused by hypersensitivity.

4.2 The Effect of EE on Systemic Metabolites

From the PCA plots of the four groups' variants, EE and P groups had more overlap in space, indicating the metabolic impaction of EE and P groups had high similarity. Metabolic clusters of 14w and N groups were far away from each other, while two administration groups were between the two normal groups with the tendency to the status of 14w group, suggesting that EE and P groups made metabolic status of 9 weeks' rats more mature, closed to 14 weeks' status.

First of all, most amino acids, like alanine, Beta-alanine, aspartate, glutamate, ornithine, serine, threonine, tyrosine and phenylalanine, were mainly up-regulated in serum and urine in EE group and increased with age. The only exception was glycine, which was significantly decreased in urine of the rats in EE and P groups. As the most important amino acid with immunoregulatory effect, threonine can not only stimulate gene express of IgA, but also participant the generation process of immunoglobulin together with serine directly (Kermanshahi et al., 2016; Su et al., 2013). 2-Hydroxybutyrate, the metabolic product of threonine, also was found up-regulated in our results. Ornithine acted as a precursor into the immune response (Mercado-Lubo et al., 2009). Glutamate can stimulate the immune system and aspartate is an incomplete degradation product of glutamate in immune cells (Yoshida et al., 1987). It has been proved that the amino acids like alanine, phenylalanine and tyrosine in the serum played an important role in the development of strengthening the immune system of children childhood (Nathalie Lepage, 1997). Alanine is a major substrate for the hepatic synthesis of glucose, a significant energy substrate for leucocytes (Wannemacher et al., 1980). Alanine is also an important component of the glycoprotein (Carletti et al., 2013; El-Sabagh et al., 2014). Tyrosine, a product of phenylalanine degradation, is the immediate precursor for the synthesis of catecholamine hormones, indicating that an increased SNS activity and an up-regulation of catecholamine metabolism followed. Norepinephrine is a key messenger released from the sympathetic nervous system to act on the immune system (Wannemacher et al., 1980). Interestingly, both Th1 cells and B cells express b2-adrenergic receptors.

Secondly, many long-chain fatty acids and their derivatives such as 9-octadecenal, 9-octadecenamide, EPA, hexadecanoate and tetradecanoate were significantly down-regulated in EE and 14w groups compared with N group in the serum. Long-chain fatty acids, especially PUFA (polyunsaturated fatty acids), had been shown to contribute to increased immune functions, such as keeping immune cells active, promoting the secretion of the type of arachate and cytokine secretion, and maintaining the integrity of the mucosal barriers (Anderson & Fritsche, 2002). In this sense, the impact of these kind of compounds on the immune system were complicated and profound, so there were also studies proving the high concentration of PUFA might possess inhibition on the immune inhibitory effect, for example, high-level EPA had an inhibitory effect on NK cells (Yamashita et al.,

1988). As representatives of polyunsaturated fatty acids, EPA and DHA acted differently in the regulation of immune functions (Gorjao et al., 2009). In this study, we can see *E. purpurea* significantly decreased the content of serum EPA in 9 weeks' rats, which was even lower than that of 14 weeks' rats, and had no effect on DHA. However, pidotimod increased the content of both the two PUFA, totally contrary to 14 weeks' results, which might be a potential causal correlation with the different impacts on IL2 and IL4.

Furthermore, some sugars and alcohols, such as threitol, glucitol, a-D-glucopyranose, were up-regulated by EE and age factor, while galactose, fructose, pinitol, ribitol and sucrose were all down-regulated. Glucose, Mannose and glycerol were significantly increased in urine in EE and P groups. From these results, it could be concluded that the impacts of *E. purpurea* and pidotimod on these metabolites were similar. It is remarkable that the decreased content of pinitol in EE and 14w groups were found, which were reported largely accumulated in antibiotic treatments (Zhao et al., 2013). Pinitol, derived from exogenous dietary crops, was believed to degrade to myo-inositol and then to acetyl-CoA via some enzymes in some bacteria (Reibach & Streeter, 1984; Streeter, 1987).

Besides pinitol, our results showed metabolites associated with gut microbiota were changed by *E. purpurea*. p-Hydroxyphenylacetate, one of the metabolites of tyramine and tyrosine produced by *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, was greatly up-regulated in urine of rats in EE and P groups (Beloborodova et al., 2012). Coincidentally, Actinobacteria phylum and *Pseudomonas* genus were increased in ileum of rats in EE groups as well. However, p-Cresol, another relevant metabolite, changed in the opposite direction. As a decarboxylation metabolite of 4-hydroxyphenylacetate by intestinal bacteria in catabolism of amino acid tyrosine and phenylalanine, p-Cresol, was considered to have negative effects on the host (Munoz-Munoz et al., 2011; Sivsammye & Sims, 1990). Substantially reduced p-Cresol could be a benefit of EE on the body. Furthermore, benzeneacetate, benzoate, hippurate, 4-hydroxycinnamate and hydrocinnamate are generated by the catabolism of natural polyphenols and flavonoids by gut bacteria (Chen et al., 2015; Sanchez-Patan et al., 2012). Some short-chain fatty acids (SCFA) also associated with gut flora, like serum propanoate, hydroxyacetate and urine Lactate, was down-regulated by EE group (van Dorsten et al., 2012). 1H-indole, a degradation product of the amino acid tryptophan by bacteria, was detected in lower level of urine in administration groups. All these variations indicated gut flora might be changed in EE and other groups.

4.3 The Effect of E. purpurea on Gut Microbiota Structure

The analysis of composition of gut microbiota demonstrated that the most dominant bacteria of all the groups belonged to two phyla, Firmicutes (Figure 5B) and Proteobacteria (Figure 5C), which are commonly founded in the mammal gastrointestinal tract. Then at genus level, several genera were present at different levels in *E. purpurea* and pidotimod-fed rats compared to control samples. *Allobaculum* and *Bifidobacterium* were increased significantly in *E. purpurea* group, while Lactobacillus was decreased significantly. The abundant genera could be clearly classified into two groups: SCFA-producing bacteria, such as *Allobaculum* and *Bifidobacterium*, and lactate-producing bacteria were decreased dramatically (Figure 5), which was in agreement with the lower level of lactic acid in urine samples (Wang et al., 2015). *Allobaculum* has been proven to be negatively correlated with obesity and could be reduced in gut by antibiotics (Cox et al., 2014).

In summary, this article studied the effects of *E. purpurea* of young rats on immune factors, systemic metabolome and gut microflora structure by comparing with pidotimod administrated young rats and adult rats. High similarity was shown in immunoregulatory cytokines and immunoglobulins, serum and urine metabolome among the three groups, indicating *E. purpurea* could promote cellular immunity and affect systemic metabolites, which might lead the juvenile body to a mature status. Metabolites of gut microbiota and the co-metabolism with host were taken together to elucidate their interaction and develop a new perspective in which *E. purpurea* can be used as a food additive for developing immunity from infectious diseases.

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